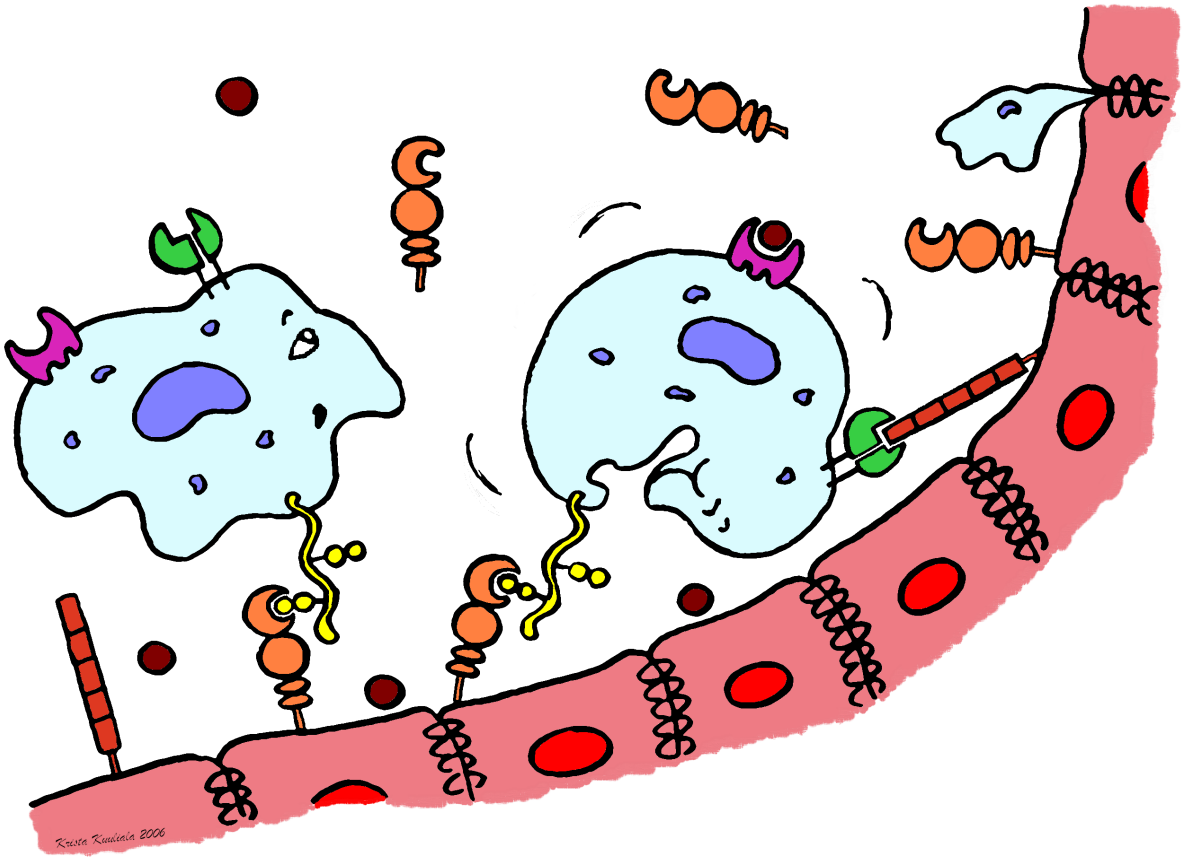
STUDIES ON IMMUNE ACTIVATION IN RHEUMATOID ARTHRITIS AND REACTIVE ARTHRITIS

Antti Kuuliala



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STUDIES ON IMMUNE ACTIVATION IN RHEUMATOID ARTHRITIS AND REACTIVE ARTHRITIS

Antti Kuuliala

Academic Dissertation

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To my family – present, dear departed, and yet to come

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II **Kuuliala A**, Takala A, Siitonen S, Leirisalo-Repo M, Repo H. Cellular and humoral markers of systemic inflammation in acute reactive arthritis and early rheumatoid arthritis. Scand J Rheumatol 33:13-18, 2004.

III **Kuuliala A**, Leirisalo-Repo M, Möttönen T, Hannonen P, Nissilä M, Kautiainen H, Korpela M, Julkunen H, Hakola M, Repo H, for the FIN-RACo Trial Group. Serum soluble interleukin-2 receptor predicts early remission in patients with recent-onset rheumatoid arthritis treated with a single disease-modifying antirheumatic drug. Clin Exp Rheumatol 23:243-246, 2005.

IV **Kuuliala A**, Nissinen R, Kautiainen H, Repo H, Leirisalo-Repo M. Circulating soluble interleukin-2 receptor level predicts rapid response in patients with refractory rheumatoid arthritis treated with infliximab. Ann Rheum Dis 65:26-29, 2006.

V **Kuuliala A**, Söderlin M, Kautiainen H, Repo H, Leirisalo-Repo M. Circulating soluble interleukin-2 receptor level predicts remission in very early reactive arthritis. Scand J Rheumatol 34:372-372, 2005.

ABBREVIATIONS

ACR	American College of Rheumatology
ARA	American Rheumatological Association
AUC	area under curve
AZA	azathioprine
CCP	cyclic citrullinated peptide
CD	cluster of differentiation
COMBI	combination DMARD treatment group
CPH82	podophyllotoxine
CRP	C-reactive protein
DAS28	28-joint Disease Activity Score
DMARD	disease-modifying antirheumatic drug
DNA	deoxyribonucleic acid
EDTA	ethylenediaminotetraacetic acid
ESL	E-selectin ligand
ESR	erythrocyte sedimentation rate
FIN-RACo	Finnish rheumatoid arthritis combination treatment study
FITC	fluorescein isothiocyanate
GST	gold sodium thiomalate
HAQ	Health Assessment Questionnaire
HCQ	hydroxychloroquine
HLA	human leukocyte antigen
ICAM	intercellular adhesion molecule
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IL-1ra	interleukin-1 receptor antagonist
IQR	interquartile range
IU	international unit
LDS-751	2-[4[4-(dimethylamino)phenyl]-1,3-butadienyl-3-ethylbenzothiazolium perchlorate
LEF	leflunomide
LPS	lipopolysaccharide
MCP	metacarpophalangeal
MMP	matrix metalloproteinase
MTP	metatarsophalangeal
MTX	methotrexate
MHC	major histocompatibility complex
n.a.	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)

n.d.	not done
NK	natural killer
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
PCT	procalcitonin
PE	phycoerythrin
PIP	proximal interphalangeal
PSGL	P-selectin glycoprotein ligand
RA	rheumatoid arthritis
ReA	reactive arthritis
RF	rheumatoid factor
RFU	relative fluorescence unit
RNA	ribonucleic acid
ROC	receiver operating characteristic
sE-selectin	soluble E-selectin
sIL-2R	soluble interleukin-2 receptor
SINGLE	single DMARD treatment group
SD	standard deviation
SpA	spondyloarthropathy
SSZ	sulphasalazine
Tc	T cytotoxic
TGF	transforming growth factor
Th	T helper
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
VCAM	vascular cell adhesion molecule
WBC	white blood cell count

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovitis, progressive joint destruction, and disability. Reactive arthritis (ReA) is a sterile joint inflammation following a distant mucosal infection. The clinical course of these diseases is variable and cannot be predicted with reasonable accuracy by clinical and laboratory markers.

The predictive value of circulating soluble interleukin-2 receptor (sIL-2R), a marker of lymphocyte activation, measured by Immulite® automated immunoassay analyzer, was evaluated in two cohorts of RA patients. In 175 patients with active early RA randomized to treatment with either on disease-modifying antirheumatic drug (DMARD) or a combination of 3 DMARDs and prednisolone, low baseline sIL-2R level predicted remission after 6 months in patients treated with a single DMARD. In 24 patients with active RA refractory to DMARDs, low baseline sIL-2R level predicted rapid clinical response to treatment with infliximab, an anti-tumour necrosis factor antibody. Furthermore, in a cohort of 26 patients with acute ReA, high baseline sIL-2R level predicted remission after 6 months.

Levels of circulating soluble E-selectin (sE-selectin), a marker of endothelial activation, were measured annually by enzyme-linked immunosorbent assay (ELISA) in a cohort of 85 patients with early RA. During a five-year follow-up, sE-selectin levels were associated with activity and outcome of RA.

The levels of neutrophil and monocyte CD11b/CD18 expression measured by flow cytometry, and circulating levels of sE-selectin measured by ELISA, and procalcitonin by immunoluminometric assay, were compared in 28 patients with acute ReA and 16 patients with early RA. The levels of the markers were comparable in ReA, RA, and healthy control subjects.

In conlusion, sIL-2R may provide a new predictive marker in early RA treated with a single DMARD and refractory RA treated with infliximab. In addition, sIL-2R level predicts remission in acute ReA.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease, which manifests as chronic polyarthritis with diverse extra-articular complications. The prevalence of RA in adult population is estimated at 0.8-1%, and the disease continues to cause significant morbidity, disability, and premature mortality. Reactive arthritis (ReA), a member of the family of spondyloarthropathies, is an acute, sterile joint inflammation developing after a variety of enteric, urogenital, or respiratory infections. The prognosis is generally good, but persistent or recurrent infection may contribute to progression into chronic spondyloarthropathy.

The clinical course of RA varies from spontaneous remission to persistent inflammation resulting in joint destruction, loss of physical function, and early death. Early treatment and achievement of remission have been shown to be of benefit. Therefore the current therapeutic strategy is to start treatment with disease-modifying antirheumatic drugs (DMARDs) in all patients. The patients refractory to DMARDs are treated with biological response modifiers, which are expensive, and associated with serious, although rare, adverse effects related to impaired host defence. Ideally, the choice of the therapy should be based on the predicted disease course and response to a given therapeutic regimen. Thus, markers are needed to identify the those patients who may achieve a good and prompt response with appropriate therapies.

The present study investigates immune activation in patients with RA and ReA, and explores markers of immune activation as tools of differential diagnosis in acute arthritides and as predictors of treatment response in RA and ReA.

REVIEW OF LITERATURE

Immune activation

The immune system is an organization of cells and molecules with specialized roles in defending against microbes and foreign cells. There are two fundamentally different types of responses to invading microbes. Innate responses occur to the same extent independent of how many times the infectious agent is encountered and provide the early protection from microbial invasion. Adaptive immunity is important later on in the immune response and improves on repeated exposure to given infection [Parkin and Cohen 2001]. Innate and adaptive responses usually work together to eliminate pathogens. Antigen presenting cells, such as dendritic cells, present antigens along with costimulatory signals to T cells, facilitating the adaptive response. Antibodies secreted by B cells, resulting from an adaptive immune response, help phagocytes ingest pathogens. Cytokines also provide an important means of communication between innate and adaptive immunity. Autoimmune disease may emerge when there is lack of immunological tolerance to self-antigens or failure to restrict or down-regulate normal immune responses [Davidson and Diamond 2001].

Innate immunity

Cellular components

The phagocytes (monocytes/macrophages and neutrophils) are the principal cell types involved in host defence. Eosinophils, basophils, and mast cells have a less defined role in innate immunity, whereas they are involved in allergic reactions [Bochner and Schleimer 2001]. Natural killer cells recognize abnormal cells, such as those infected with a virus, inducing apoptosis [Yokoyama *et al.* 2004].

Monocytes and macrophages

Monocytes are the circulating precursors of tissue macrophages. They lack phagocytic capacity and are relatively inert whilst in the blood compartment. Once the monocyte leaves the circulation it differentiates into the macrophage, defined by its capacity to phagocytose. The phenotypic form taken by the macrophage depends on the environmental factors present in the tissue [Duffield 2003].

Classically activated macrophages are developed in response to interferon-γ (IFN-γ), along with exposure to bacterial lipoproteins, bacterial DNA, parasitic proteins/carbohydrates,

opsonized particles, or exogenous tumor necrosis factor (TNF). Also ligation of chemokine receptors, hypoxia, and abnormal collagen deposition push the macrophage towards classical activation phenotype. IFN-γ augments cytokine and toxic nitrogen radical release, and up-regulates expression of receptors necessary for effective presentation of antigens to the adaptive immune system [Mosser 2003].

Exposure to IL-4, IL-10, IL-13, transforming growth factor-β (TGF-β), or glucocorticoids causes macrophages to become alternatively activated, preventing the classical inflammatory phenotype. These macrophages generate anti-inflammatory cytokines, while the synthesis of pro-inflammatory cytokines is suppressed. However, they show enhanced capacity for antigen presentation and enhanced phagocytosis of debris and particles, though not pathogens [Mosser 2003].

Neutrophils

Neutrophils are mobile cells that travel around the body. They normally flow freely in the blood or roll along the vascular endothelium (marginating pool). To home to a site of infection, neutrophils use a multistep process involving proinflammatory mediators, adhesion molecules, chemoattractants, and chemokines [Ebnet and Vestweber 1999].

The recruited neutrophils phagocytose organisms by making pseudopodia (projections of cytoplasmic membrane) which form a membrane-bound vesicle (phagosome) around the particle. This fuses with cytoplasmic granules to form the phagosome. In this protected compartment, killing of the organism occurs by a combination of two mechanisms. The oxygen-dependent response or respiratory burst involves the sequential reduction of oxygen by NADPH oxidase leading to production of toxic oxygen metabolites, such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen. The oxygen-independent response uses the highly toxic cationic proteins and enzymes (e.g., myeloperoxidase and lysozyme) contained within the neutrophil cytoplasmic granules. Ingestion and killing of organisms is 100-fold more effective if the particle is first opsonised with specific antibody or complement. These molecules bind to neutrophil Fc and complement receptors, increasing adhesion between particle and phagocyte and priming the cell for activation [Witko-Sarsat *et al.* 2000].

Soluble components

Cytokines

Cytokines are small molecular weight messengers secreted by one cell to alter the behaviour of itself or another cell (Figure 1). The biological effect of a cytokine depends on the cytokine and the cell involved and its environment, but typically they affect cell activation, division, apoptosis, or movement. Cytokines bind to specific receptors. Most cytokines are soluble, but

some may also be membrane-bound. Cell-surface cytokine receptors may also be secreted in soluble form [Borish and Steinke 2003].

Cytokines produced by leukocytes and having effects mainly on other leukocytes are termed interleukins. Over 30 interleukins have been currently described. Cytokines that have chemoattractant activity are called chemokines. Those that cause differentiation and proliferation of stem cells are called colony-stimulating factors. Those that interfere with viral replication are called interferons.

TNF is mainly produced by monocytes/macrophages, but also by neutrophils, activated lymphocytes, NK cells, endothelial cells, and mast cells. The most potent inducer of TNF by monocytes is LPS. TNF has two distinct receptors: TNFR I (p55) and TNFR II (p75). They have similar affinities and produce similar effects [Tartaglia and Goeddel 1992]. TNF induces antitumour immunity through direct cytotoxic effects on cancerous cells, hence the name tumour necrosis factor [Carswell *et al.* 1975]. On the whole, TNF stimulates general immune activation. TNF induces adhesion molecules (intercellular adhesion molecule-1, ICAM-1; vascular cell adhesion molecule-1, VCAM-1; E-selectin) on endothelial cells, permitting adhesion and subsequent transmigration of leukocytes. TNF activates neutrophils, mediating adherence, chemotaxis, degranulation and respiratory burst [Vassalli 1992]. TNF induces vascular leakage, and is also responsible for the severe cachexia (hence the previous name cachexin), which occurs in chronic infections and cancer [Beutler and Cerami 1989].

IL-1 family comprises four peptides: IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra), and IL-18. IL-1 is primarily produced by monocytes/macrophages, but also by endothelial cells, keratinocytes, synovial cells, osteoblasts, neutrophils, glial cells, and numerous other cell types. IL-1 production can be stimulated by a variety of agents, including lipopolysaccharide (LPS), other cytokines, microorganisms, and antigens. IL-1 has two types of receptors (IL-1R), type I IL-1R responsible for the proinflammatory effect of IL-1, and the inactive type II IL-1R thought to sequester IL-1 and thus have an anti-inflammatory function [Dinarello 1998].

IL-1 activates T lymphocytes by enhancing the production of IL-2 and expression of IL-2 receptors (IL-2R). It also augments B-cell proliferation and immunoglobulin synthesis. IL-1 interacts with the central nervous system to produce fever, lethargy, sleep, and anorexia [Licinio and Wong 1997]. IL-1 stimulates endothelial cell adherence to leukocytes through the upregulation of adhesion molecules (ICAM-1, VCAM-1, E-selectin). IL-1ra binds proinflammatory IL-1 receptors without causing intracellular signaling, thus acting as a modulator of inflammation [Dinarello 1998].

IL-6 is mainly produced by monocytes/macrophages, as well as T and B lymphocytes, fibroblasts, endothelial cells, keratinocytes, hepatocytes, and bone marrow cells. It facilitates the differentiation of B lymphocytes to plasma cells. IL-6 mediates T cell activation, growth,

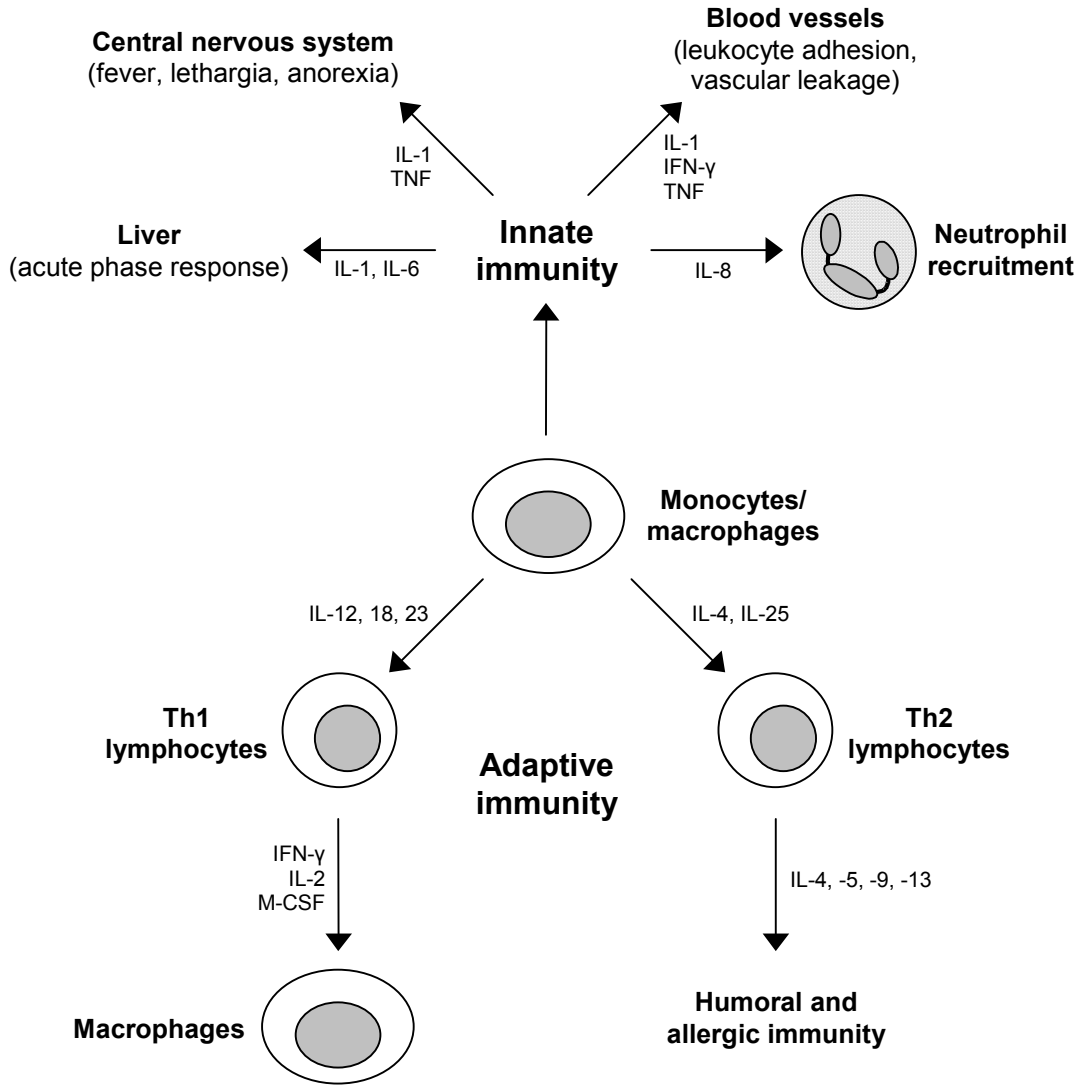

Figure 1. Summary of cytokine actions. Cytokines are derived predominantly from monocytes/macrophages. They are uniquely important in initiating innate immunity responses. The phenotype of the subsequent immune response is a function of the repertoire of cytokines produced by the responding T-helper lymphocytes. Adapted from Borish and Steinke [2003].

and differentiation. IL-6 is considered to be the most important inducer of hepatocyte synthesis of acute phase proteins. IL-6 also has several anti-inflammatory effects, for example stimulating the synthesis of IL-1ra. IL-6 inhibits the synthesis of TNF and IL-1, both of which induce synthesis of each other and IL-6 [Akira *et al.* 1993].

Acute phase proteins

An acute phase protein has been defined as one whose plasma concentration increases (positive acute phase proteins) or decreases (negative acute phase proteins) by at least 25% during inflammatory disorders. Conditions that substantially influence acute-phase proteins

include infection, trauma, advanced cancer, and rheumatic diseases and other immunologically mediated conditions [Gabay and Kushner 1999].

The changes in the concentrations of acute phase proteins are largely due to their changes in their production by hepatocytes. IL-6 is the chief stimulator of the production of most acute-phase proteins [Akira *et al.* 1993]. Different cytokine combinations have been found to have additive, inhibitory, or synergistic effects. The expression of acute phase protein genes is mainly regulated at the transcriptional level, but post-translational mechanisms, such as modulation of messenger RNA stability and translation, also play a role [Gabay and Kushner 1999].

C-reactive protein is currently the most widely used measurement used in evaluating the acute phase response. It is able to bind to phosphocoline and thus recognize some foreign pathogens as well as phospholipids of damaged cells, and activate the complement system when bound to one of its ligands. Other proinflammatory effects include the induction of inflammatory cytokines and tissue factor in monocytes. CRP may also have an anti-inflammatory effects, by preventing neutrophil adhesion to endothelial cells, inhibiting superoxide generation by neutrophils, and stimulating the synthesis of IL-1ra by mononuclear cells [Du Clos and Mold 2004].

Procalcitonin is a precursor of the hormone calcitonin, secreted by the thyroid gland. Normally the procalcitonin levels in the circulation are very low. However, very high levels are detected in sepsis [Meisner 2002], also in patients who have previously undergone thyroidectomy [Assicot *et al.* 1993]. The source of inflammatory procalcitonin is unknown. It is thought to originate from the liver [Nijsten *et al.* 2000], but also mononuclear cells express procalcitonin [Oberhoffer *et al.* 1999].

Adaptive immunity

Adaptive immunity uses antigen-specific receptors on T and B cells to drive targeted effector responses in two stages. First, the antigen is presented to and recognized by the antigen specific T or B cell leading to cell priming, activation and differentiation, which usually occur within the specialised environment of lymphoid tissue. Second, the effector response takes place, either by activated T cells that leave the lymphoid tissue and migrate to the disease site, or by activated B cells (plasma cells) that release antibodies into the blood and tissue fluids, and thence the infective focus [Parkin and Cohen 2001].

B and T lymphocytes develop from progenitor cells within the bone marrow. B cells remain within the marrow for the duration of their development, but T cells migrate to the thymus at and early stage as thymocytes. The production of antigen-specific receptors in both cell types is the result of an unusual process of random rearrangement and splicing together of multiple

DNA segments that code for the antigen-binding areas of the receptors (complementarity-determining regions). Gene rearrangement occurs early in the development of the cells, before exposure to antigen, which leads to the production of a repertoire of over 10^8 T-cell receptors and 10^{10} antibody specificities, adequate to cover the range of pathogens likely to be encountered in life. The creation of new clones of T and B cells continues through life [Nemazee 2000].

The cells that have undergone gene rearrangement and emerge from the thymus and bone marrow are naïve, i.e., they have not yet encountered their specific antigen within an immune response. Consequently, there are only a few naïve T and B cells capable of recognizing a foreign particle and there has to be a system to bring the naïve cells and their targets together. The secondary lymphoid tissues of the lymph nodes, spleen, tonsils, and mucosal membranes provide the microenvironment for this process, and naïve cells migrate to them. In addition to T and B lymphocytes, they contain efficient antigen-presenting cells, and lymphocytes are provided with cytokines for maintenance and the second signal that is necessary when lymphocyte activation takes place [Guermonprez *et al.* 2002].

T lymphocytes

Antigens are presented to T cells in association with a self-MHC molecule. The delicate process of positive selection of T cells that can react with self-MHC and peptide adequately to induce immune responses, but are not excessively MHC-reactive to the extent that would cause self-tissue destruction, occurs in the thymus [Parkin and Cohen 2001]. The need for expression with MHC ensures that only antigens derived from foreign molecules that have invaded the host cell or induced an inflammatory response to activate endocytosis by antigen-presenting cells, are recognized as foreign. In addition, simultaneous signalling via costimulatory molecules is required to induce activation of the T cell [Mueller 2000]. Inflammatory mediators induce the upregulation of costimulatory molecules. Therefore a T cell is much more likely to be activated if it meets its specific antigen via an antigen-presenting cell, which has been exposed to an inflammatory environment.

T helper (Th) cells expressing CD4 are the orchestrating cells of the immune response, recognising foreign antigen, and activating other parts of the cell-mediated immune response to eradicate the pathogen. They also play a major part in activation of B cells. Th lymphocytes only recognise exogenous antigen taken up and processed by professional antigen presenting cells and presented with MHC class II. Antigen-presenting cells include dendritic cells (the interdigitating dendritic cells of the lymph nodes, veiled cells in the blood, and Langerhans cells in the skin), B cells, and macrophages [Sprent 1995].

On stimulation, precursor Th0 lymphocytes become either Th1 or Th2 cells [Swain *et al.* 1991]. Th1 cells produce IL-2, which induces T cell proliferation, including that of CD4+

cells in an autocrine fashion. IL-2 also stimulates CD8+ T cell division and cytotoxicity. The other major cytokine produced by Th1 cells, IFN-γ, activates macrophages to kill intracellular pathogens such as mycobacteria, fungi, and protozoa and induces natural killer cells to cytotoxicity. The Th1 cytokines therefore induce mainly a cell-mediated inflammatory response. There is a positive feedback loop as IFN-γ stimulates other Th0 cells to become Th1 and inhibits Th2 differentation. A Th1 response is essential to the host to control the replication of intracellular pathogens, but it possibly contributes to the pathogenesis of autoimmune disease such as RA. Conversely, Th2 cells produce IL-4, -5, -6, and -10, that favour antibody production. IL-4 induces class switching in B cells leading to IgE production, and IL-5 promotes positive feedback to induce further Th2 responses and suppress Th1 differentiation. Thus the Th2 response is associated with allergic disease.

Cytotoxic T cells (Tc) expressing CD8 are involved in antiviral and possibly antitumour activity. Endogenous antigens complexed with MHC class I molecules activate Tc cells. Because all nucleated cells express MHC class I, any such cell that is infected with a virus or other intracellular pathogen, or is producing abnormal tumour antigens can present these antigens with class I and be removed by cytotoxic attack. After binding to the target cell, Tc cells release cytoplasmic granules at the contact site. these granules contain perforin that forms pores to the target cell plasma membrane and granzymes that pass through the pores into the target cytoplasm. These enzymes activate caspases that induce DNA fragmentation and cell apoptosis. Receptor-mediated apoptosis is also involved, as Tc bind target cell surface Fas (death inducing) molecules by their Fas ligand [Alam and Gorska 2003].

B lymphocytes

Most B cells remain in the lymphoid tissue and recognise free antigen brought there. However, during subsequent infections by the same pathogen B cells can be activated by follicular dendritic cells which bear Fc and complement receptors, bind immune complexes containing antigen, and trap this to activate the B cell response.

B cells produce antibodies [Edelman 1973]. They neutralise toxins, prevent organisms from adhering to mucosal surfaces, activate complement, opsonise bacteria for phagocytosis, and sensitise tumour and infected cells for antibody-dependent cytotoxic attack by killer cells. Thus, antibodies act partly by enhancing elements of innate immunity. The so-called natural antibodies play an important role in this context. They are IgM class antibodies secreted without antigenic stimulation, considered to provide a first line of defence against invading pathogens [Baumgarth *et. al.* 2005]. They are often autoreactive, and may also play a role in recognition and removal of senescent cells, cell debris, and other self-antigens. Although ultimately antibody is the secreted product of activated B cells with the functions listed above, early in B cell development it is a membrane-bound molecule that acts as the B cell receptor.

In this role the B cell internalises antigen and processes it to act as an antigen-presenting cell for T cell responses.

Antigen recognised by the surface IgM of the B cell is internalised, processed, and re-expressed on the MHC class II molecule of the B cell. This can then present the antigen to a primed specific T cell (which recognises a different part of the same antigen). The T cell in turn produces cytokines leading to B cell division and maturation to antibody secreting cells [Lanzavecchia 1990]. Further T cell interactions induce isotype switching from the initial IgM response. Somatic hypermutation occurs, leading to greater antibody diversity. Those cells whose surface antibody binds the antigen most avidly proliferate most efficiently. Therefore the antibody response matures with increased affinity. The activated B cells leave the lymphoid tissue as plasma cells. Some of the activated cells become long-lived memory cells. These react rapidly to rechallenge and the characteristic IgG production of the secondary response occurs.

Soluble interleukin-2 receptor

IL-2 is made by T cells, some B cells and dendritic cells. The major function of IL-2 is to promote proliferation of both CD4+ and CD8+ T cells. T cells activated through their antigen receptors produce IL-2 and the high affinity IL-2 receptor, thus permitting rapid and selective expansion of effector T cell populations. This is achieved through proliferative and anti-apoptotic mechanisms [Gaffen and Liu 2004].

The interleukin-2 receptor (IL-2R) is composed of three subunits (Table 1). IL-2Rα (also known as CD25 or Tac) constitutes the low affinity receptor, which enhances the affinity of IL-2R for ligand by approximately 100-fold but does not contribute to signal transduction. IL-2Rβ (p75) and IL-2Rγ (γc, p65) subunits are necessary and sufficient for effective signalling. Neither IL-2Rβ nor IL-2Rγ alone binds IL-2 detectably, but the IL-2Rβ/IL-2Rγ complex comprises the intermediate affinity IL-2 receptor, which is capable of mediating the full spectrum of IL-2-dependent activities. IL-2Rβ and IL-2Rγ subunits are employed in other cytokine receptors, but sIL-2Rα is exclusive to the IL-2R [Gaffen 2001].

A soluble form of IL-2Rα is released upon cell activation. Soluble interleukin-2 receptor (sIL-2R) is a marker of immune activation [Rubin and Nelson 1990]. It is considered to reflect

Table 1. Compositions of IL-2 receptor complexes.

IL-2 affinity	High	Intermediate	Low
Subunit composition	αβγ	βγ	α
Ability to signal	Complete	Complete	None

mostly T cell activation, and it has been suggested that sIL-2R particularly reflects a Th1 type response [Berghella *et al.* 1998].

sIL-2R is present in low amounts in sera of healthy individuals. Elevated levels have been detected in various clinical conditions, including haematological malignancies, infections and autoimmune diseases [Rubin and Nelson 1990].

Systemic inflammation

During the very early stages of infection or tissue damage activated macrophages release cytokines. Two of these, granulocyte and granulocyte-macrophage colony stimulating factors, stimulate division of myeloid precursors in the bone marrow, releasing millions of cells into the circulation and causing a characteristic neutrophil leucocytosis. The activation of complement generates C3b, which coats the pathogen surface. The neutrophil chemoattractant and activator C5a is also produced, and together with C3a and C4a it triggers histamine release by mast cells degranulation. This in turn causes the contraction of smooth muscles and rapid increase in local vascular permeability.

Substances released from the pathogen and from damaged tissues up-regulate the expression of adhesion molecules on vascular endothelium, alerting passing cells to the presence of infection (Figure 2). The cell-surface molecule L-selectin on neutrophils recognizes carbohydrate structures such as sialyl-Lewis^x on the vascular adhesion molecules. The neutrophil rolling along the vessel wall is arrested by these interactions. As the neutrophil becomes activated, it rapidly sheds L-selectin from its surface and replaces it with integrins and other cell-surface adhesion molecules. Integrins bind E-selectin, which appears on the blood vessel wall under the influence of inflammatory mediators such as bacterial LPS and

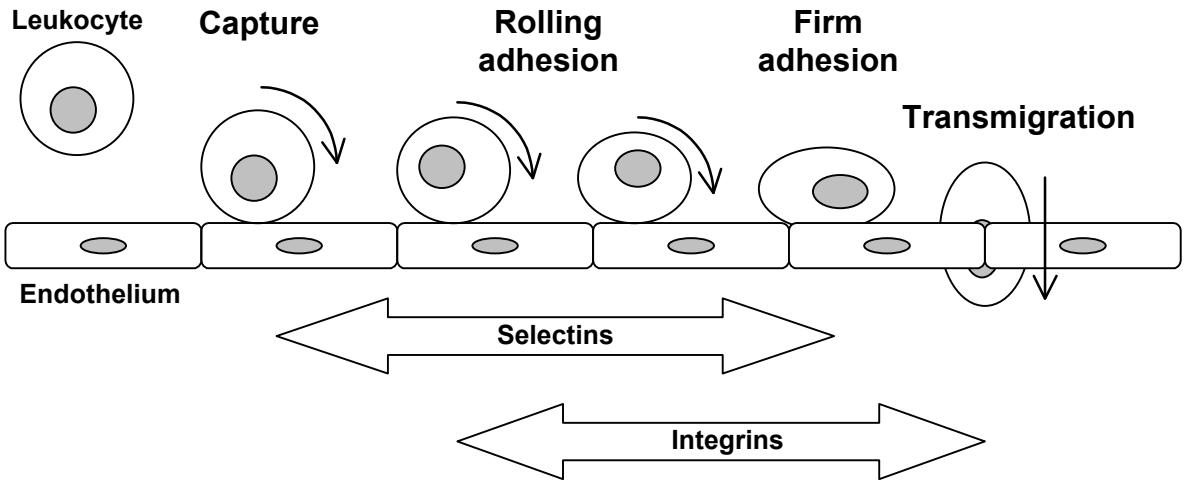

Figure 2. The leukocyte adhesion cascade.

the cytokines IL-1 and TNF [Repo and Harlan 1999]. Complement components, prostaglandins, leukotrienes, and other inflammatory mediators contribute to the recruitment of inflammatory cells, as does an important group of chemoattractant cytokines called chemokines. The activated neutrophils pass through the vessel walls, moving up the chemotactic gradient to accumulate at the site of infection, where they are placed to phagocytose any C3b-coated microbes [Ebnet and Vestweber 1999].

The rolling step is mediated by neutrophil L-selectin and by E- and P-selectins newly expressed on inflamed endothelial cells. P-selectin, mobilized to the endothelial cell surface in a few minutes following stimulation by thrombin, histamine, or oxygen radicals, interacts primarily with P-selectin glycoprotein ligand-1 (PSGL-1). Rolling subsequently involves E-selectin, which appears on endothelial cells one to two hours after stimulation by IL-1, TNF, or LPS. E-selectin counter-receptors include PSGL-1 and E-selectin ligand-1 [Ebnet and Vestweber 1999]. E-selectin is shed from the endothelial surface in a soluble form (soluble E-selectin, sE-selectin), which can be measured as a marker of systemic inflammation [Gearing and Newman, 1993].

Several integrins are implicated in the adhesion of leukocytes to the endothelium and migration in the inflamed tissue. Granulocytes and monocytes express CD11b/CD18, which upon cell activation undergoes a conformational change resulting in increased affinity. Additional CD11b/CD18 molecules are also rapidly translocated to the cell surface from intracellular vesicles. Thus, the cell surface expression of CD11b/CD18 can be used as a marker of phagocyte activation and systemic inflammation [Repo and Harlan 1999].

Rheumatoid arthritis

Definitions and clinical features

RA is a chronic systemic inflammatory disease which targets synovial joints, and is often accompanied by an array of extra-articular manifestations [Lee and Weinblatt 2001]. RA occurs worldwide, increases in incidence with age, and affects women approximately 3 times more often than men. The prevalence of RA is estimated at 0.8-1.0% in adult population, and the disease continues to cause significant morbidity, disability, and premature mortality.

The presentation and course of RA is variable. Patients most often have an insidious onset of symmetrical joint pain, swelling, and morning stiffness that worsens over several weeks. Generalized malaise and fatigue accompany active inflammation.

Physical findings in RA include symmetrical joint inflammation early in the course of the disease and manifestations of progressive joint destruction with chronic disease. Warmth,

Table 2. The American College of Rheumatology classification criteria of rheumatoid arthritis. For the diagnosis of RA, a patient should have at least 4 of the 7 criteria. Criteria 1-4 must have been present for at least 6 weeks.

1. Morning stiffness (1 hour or more)
2. Arthritis of 3 or more joint areas
3. Arthritis of hand joints (PIP, MCP, wrist)
4. Symmetric arthritis
5. Rheumatoid nodules
6. Serum rheumatoid factor
7. Radiographic changes in hand and/or wrist joint

PIP, proximal interphalangeal joint; MCP, metacarpophalangeal joint.

swelling, pain, reduced range of motion, and palpable effusions characterise active synovitis. Classically, RA causes synovitis in the metacarpophalangeal (MCP) joints and proximal interphalangeal (PIP) and metatarsophalangeal (MTP) joints in a symmetrical distribution. RA commonly affects the feet, wrists, and knees, as well as the cervical spine, glenohumeral joints, and hips.

Extra-articular manifestations of RA include subcutaneous rheumatoid nodules, vasculitic skin ulceration, sicca symptoms, pulmonary nodules and pulmonary interstitial fibrosis, mononeuritis multiplex, and Felty's syndrome [Turesson *et al.* 1999].

The American College of Rheumatology (ACR) has provided classification criteria of RA (Table 2) [Arnett *et al.* 1988].

Pathogenesis

Immunological changes

Signs of an aberrant immune response, *e.g.* rheumatoid factor seropositivity, appear many years before the onset of clinical symptoms [Aho *et al.* 1985].

Histological changes

The inflamed synovium shows pronounced angiogenesis, cellular hyperplasia, influx of inflammatory leukocytes, and changes in the expression of cell surface adhesion molecules, proteinases, proteinase inhibitors, and many cytokines. Synovial changes in the rheumatoid joint vary with disease progression [Hitchon and el-Gabalawy 2003]. In the first weeks of the disease, tissue oedema and fibrin deposition are prominent and can manifest clinically as joint swelling and pain. Within a short period, the synovial lining becomes hyperplastic, commonly

becoming ten or more cells deep and consisting of type A (macrophage-like) and type B (fibroblast-like) synoviocytes. The sublining also undergoes striking alterations in cellular number and content, with prominent infiltration of mononuclear cells, including T cells, B cells, macrophages, and plasma cells. Synovial-vessel endothelial cells transform into high endothelial venules early in the course of the disease.

The formation of locally invasive synovial tissue – pannus – is a characteristic feature of RA. This tissue is involved in the joint erosions seen in RA [Gravallese 2002]. Pannus is histologically distinct from other regions of the synovium and shows phases of progression. Initially, there is penetration of the cartilage by synovial pannus composed of mononuclear cells and fibroblasts with high-level expression of matrix metalloproteinases (MMPs) by synovial lining cells. In later phases of the disease, cellular pannus can be replaced by fibrous pannus comprised of a minimally vascularized layer of pannus cells and collagen overlying cartilage.

Cellular mediators

The synovial membrane is infiltrated by inflammatory cells, primarily CD4+ T cells. The predilection for HLA-DR alleles suggests that RA is caused by an unidentified arthritogenic antigen [Weyand and Goronzy 1997]. The antigen could be either an exogenous antigen, such as viral protein, or an endogenous protein.

Antigen-activated CD4+ T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce the cytokines IL-1, IL-6, and TNF and to secrete MMPs through cell-surface signalling as well as the release of soluble mediators such as IFN-γ and IL-17. Activated CD4+ T cells also stimulate B cells to produce immunoglobulins, including rheumatoid factor. The precise pathogenic role of rheumatoid factor is unknown, but it may involve the activation of complement through the formation of immune complexes. Activated CD4+ T cells express ostroprotegerin ligands that stimulate osteoclastogenesis (Figure 3).

Activated macrophages, lymphocytes, and fibroblasts, as well as their products, can also stimulate angiogenesis, which may explain the increased vascularity found in the RA synovium. Endothelial cells in the synovium are activated and express adhesion molecules, such as E-selectin, that promote the recruitment of inflammatory cells into the joint. This process is enhanced by the release of chemokines, such as IL-8, by inflammatory cells in the joint [Choy and Panayi 2001].

Soluble mediators

Monocytes, macrophages, fibroblasts, and T cells release numerous cytokines on stimulation. TNF and IL-1 are likely to have primary roles in the pathogenesis of RA. The serum and synovial concentrations of both cytokines are high in patients with active RA. Furthermore, TNF and IL-1 stimulate the release of MMPs and inhibit the production of tissue inhibitors of

MMPs, leading to joint damage. TNF stimulates the development of osteoclasts, which are responsible for bone degradation (Figure 3).

Joint damage

RA is characterized by progressive joint damage that is mediated by several mechanisms [Gravallese 2002]. Early erosion of cartilage and bone is associated with the formation of a proliferating pannus. The interface between pannus and cartilage is occupied predominantly by activated macrophages and synovial fibroblasts that express MMPs and cathepsins.

IL-1 and TNF stimulate the expression of adhesion molecules on endothelial cells and increase the recruitment of neutrophils into the joints. Neutrophils release elastase and protease, which degrade proteoglycan in the superficial layer of cartilage. The depletion of proteoglycan enables immune complexes to precipitate in the superficial layer of collagens and exposes chondrocytes. When stimulated by IL-1, TNF, or activated CD4+ T cells, chondrocytes and synovial fibroblasts release MMPs, which are thought to be the main mediators of joint damage. Activated CD4+ T cells may also stimulate osteoclastogenesis.

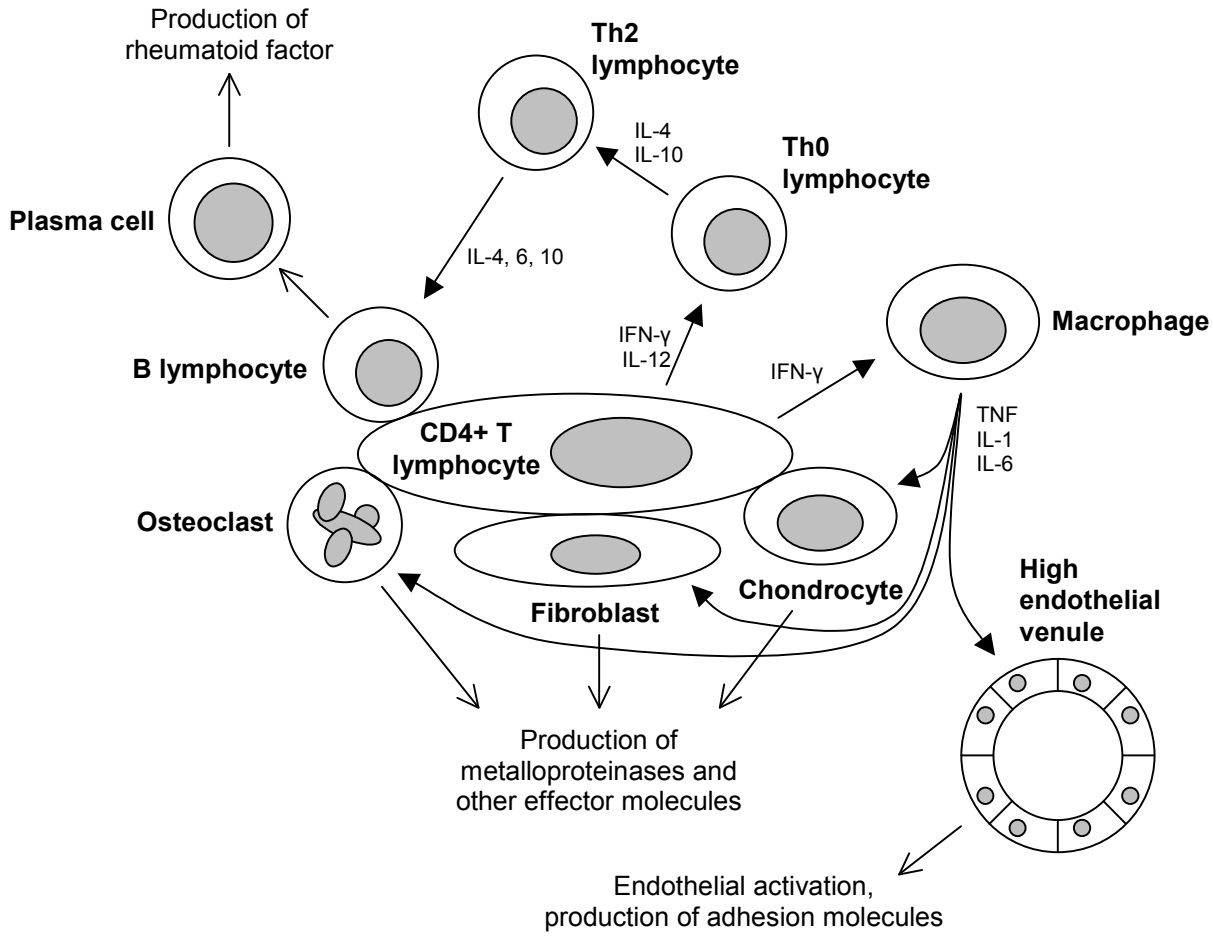

Figure 3. Cytokine signaling pathways involved in inflammatory arthritis. Adapted from Choy & Panayi [2001].

Treatment

The goals of treatment in RA are to control inflammation, prevent progressive joint destruction, preserve and improve activities of daily living, and alleviate pain. Medical treatment includes the use of non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), corticosteroids, and biological response modifiers. In addition to drug therapy, nonpharmacological treatment, including patient education, physiotherapy, occupational therapy, orthotics, and surgery are important.

Disease-modifying antirheumatic drugs

A major transformation in the pharmacological treatment of RA occurred in the 1990s. The previous therapeutic approach, termed as the therapeutic pyramid, generally involved initial conservative management with NSAIDs for several years; DMARDs were withheld until clear evidence of the progressive character of the disease in the face of erosions was seen. DMARDs were then added individually in slow succession if the disease progressed. This form of treatment has been replaced by a) early initiation of DMARDs and b) use of combinations of DMARDs in patients with the potential for progressive disease. The idea of early intervention with DMARDs has been validated in a randomised trial [Möttönen *et al.* 2002].

Methotrexate has become the most widely prescribed DMARD. Other commonly utilized DMARDs include leflunomide, sulphasalazine, and hydroxychloroquine. Also cyclosporine, azathioprine, D-penicillamine, and gold salts remain in use. DMARD monotherapy is often only partly effective and poorly tolerated in long term. DMARD combination therapy has been explored as a way to maximize therapeutic effect while maintaining a tolerable toxic-effect profile. Initial randomized controlled trials of monotherapy versus combination therapy yielded conflicting results. However, more recently it has been shown that combination therapy has clear benefits and tolerable toxic effects in both early and chronic RA. In general, the beneficial combinations have included methotrexate and 1 to 3 other DMARDs [Pincus *et al.* 1999].

Biological response modifiers

Biological response modifiers, whose effects are based on directly manipulating cytokines central to the pathogenesis of RA, have become an important new class of RA therapeutics [Goldblatt and Isenberg 2005]. Currently three biological response modifiers targeting TNF are in clinical use. Two of them are monoclonal anti-TNF antibodies. Infliximab is a chimeric IgG1 antibody consisting of the antigen-binding region of mouse antibody and the constant region of the human antibody, and adalimumab is a recombinant human IgG1 antibody. They bind soluble and membrane-bound TNF, thereby impairing binding of TNF to its receptor, and also mediate killing of cells expressing TNF. Etanercept is a soluble TNF-receptor fusion

protein, composed of 2 dimers, each with the ligand binding portion of p75 receptor linked to the Fc portion of human IgG1. The protein binds TNF, preventing it from engaging its receptor. In addition, currently in clinical use is anakinra, a recombinant form of IL-1 receptor antagonist.

Other promising biological response modifiers not yet in clinical use in rheumatology include abatacept, rituximab, and MRA. Abatacept (CTLA4Ig) is a costimulation blocker, consisting of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) fused to the heavy chain region of human IgG1. Rituximab is a chimeric anti-CD20 monoclonal antibody, which selectively depletes B cells by binding to CD20. MRA is a humanized anti-IL-6 receptor antibody, which inhibits binding of IL-6 to the IL-6 receptor [Doan and Massarotti 2005]. Biological response modifiers developed but abandoned due to inadequate clinical benefit or excessive toxicity include monoclonal antibodies against ICAM-1, CD4, and IL-2 receptor, recombinant anti-inflammatory cytokines, such as IL-4, IL-10, and IL-11, and matrix metalloproteinase inhibitors [Keystone 2003].

Biological response modifiers are generally well tolerated and often yield a response even in patients refractory to traditional DMARDs. However, multiple adverse events are attributed to the biological response modifiers. Also, they are very expensive. Patients treated with anti-TNF agents are at increased risk of serious infections, most notably reactivation of latent tuberculosis, but also listeriosis, *Pneumocystis carinii* pneumonia, histoplasmosis, aspergillosis, and severe *Candida* infections [Keane *et al.* 2001]. There is also concern that long-term use of anti-TNF drugs may increase the risk of cancer, particularly lymphoma.

Prognostic factors

The two main complications of RA are joint destruction and disability. The search for prognostic factors has mostly been focused on prediction of joint destruction [Morel and Combe 2005, Scott 2000].

Genetic factors are the ideal prognostic markers because they are present at disease onset and are unchanged by treatment. Several genes have been studied in RA. The presence of the shared epitope, *i.e.* an identical short amino acid sequence found in several HLA-DR alleles, has been suggested to predict erosive disease. However, the role of genetic markers in the prognosis of RA remains unclear and they are too insensitive to be applied in clinical practice as guidance in the choice of therapy.

Environmental factors, such as socio-economic status, level of formal education, age at disease onset, and lifestyle factors such as smoking, have been implicated in affecting the outcome of early RA. However, the results are conflicting and therefore these factors are not reliable prognostic factors in early RA.

Disease activity at onset is fundamental with respect to the prognosis of RA. In early RA, the number of swollen joints predicts joint damage. High erythrocyte sedimentation rate and circulating C-reactive protein level are correlated with radiological outcome.

Rheumatoid factor (RF) is associated with poor prognosis in early RA. Seropositive patients have a more progressive disease compared to seronegative patients. High level of RF predicts more destructive disease.

Other laboratory markers, such as anti-perinuclear neutrophil cytoplasmic antibodies, antikeratin antibodies, and anti-cyclic citrullinated peptide antibodies have been shown to predict radiographic progression.

Radiological damage at baseline represents the best predictive factor of severe outcome. The initial radiographic score consistently predicts radiological damage by approximately 3 years [Combe *et al.* 2001].

Disability at onset measured with the Stanford Health Assessment Questionnaire (HAQ) is the most powerful predictor of functional impairment, work disability, and mortality. During the first years of the disease, HAQ is associated with clinical inflammation, but later in the course of the disease it is also associated with radiological damage.

Clinical remission is the goal of treatment of RA. There are only a few studies that have also sought to find prognostic factors for remission. Low disease activity score, HAQ score, Ritchie score, and CRP level at baseline were found to predict remission at 3 years in early RA [Gossec *et al.* 2004]. Also the absence of anti-cyclic citrullinated peptide (CCP) antibodies and RF at onset has been found to be associated with remission [Bas *et al.* 2003a].

However, currently there are no prognostic markers reliable enough for clinical decision-making. The prognostic markers mentioned above predict disease severity in patient cohorts, but not in a single patient.

Reactive arthritis

Definitions and clinical features

Reactive arthritis (ReA) is defined as a sterile synovitis developing after a distant infection, usually in the genitourinary or gastrointestinal tract. It belongs to the group of spondyloarthropathies (SpA), which also includes ankylosing spondylitis, psoriatic arthritis,

arthritis in inflammatory bowel disease, and undifferentiated SpA [Stafford and Youssef 2002].

A number of microbes can trigger ReA. These infections affect mostly the mucosal surfaces in the gastrointestinal (*Yersinia*, *Salmonella*, *Shigella*, *Campylobacter*), urogenital (*Chlamydia trachomatis*) or respiratory tract (*Chlamydia pneumoniae*).

Most commonly the large joints of the lower extremities are affected, but any joint may be inflamed. Inflammatory low back pain and stiffness frequently occur. Entesopathies, tenosynovitis, or both, are present in a high proportion of cases. The severity of the arthritis varies from mild and transient swelling or discomfort to febrile, severely invalidating conditions, which may be hard to differentiate from pyogenic arthritis [Toivanen and Toivanen 2004].

Pathogenesis

The microbes that cause ReA (*Yersinia*, *Salmonella*, *Shigella*, *Campylobacter*, and *Chlamydia*) are gram-negative, intracellular pathogens and contain LPS. They cause the primary infection in mucosal tissue, and enter the body. A complex host-microbe interaction begins. CD4+ T cells are activated and sensitized to bacterial antigens in the intestinal epithelium [Sieper and Braun 1995].

The joint cultures are negative for triggering microbes. However, there is evidence that bacterial antigens circulate from the focus of infection to the joints. Bacterial LPS, RNA, and DNA are transported to the joints inside phagocytes. The antigens stimulate the sensitized CD4+ T cells, which are mostly of the Th2 type in ReA. It can be assumed that a Th1 type response would lead to elimination of the pathogens and suppression of arthritis, whereas a Th2 type response leads to persistence of microbes and prolonged or chronic arthritis.

HLA-B27 is the strongest predisposing factor for ReA. About 50-80% of patients in hospital based series are HLA-B27 positive [Leirisalo *et al.* 1982]. However, at population level, the frequency is considerably lower [Hannu *et al.* 2002]. Several theories have been suggested about the role of HLA-B27 in the pathogenesis of ReA [Toivanen and Toivanen 2004]. HLA-B27 may enhance the invasion of bacteria or inhibit the killing of intracellular bacteria. HLA-B27 may also present bacterial antigens in such ways that lead to autoimmunity. However, the role of HLA-B27 in ReA remains unclear.

Treatment

Treatment of ReA should be directed at relief of pain, suppression of inflammation, maintenance of function, optimal joint protection, and, when appropriate, eradication of infection. Patient education about the prognosis and treatment is essential. Joint rest may be needed to alleviate pain in severe cases, but generally inactivity and immobilization should be discouraged. Physical therapy can be used to regain muscle strength and range of motion.

NSAIDs form the cornerstone of the treatment, providing analgesic and anti-inflammatory effects, and should be used over an extended period of time. Corticosteroids may be used as intra-articular injections or perorally for short periods of time in severe joint inflammation. Treatment with DMARDs should be considered in severe cases of ReA, where polyarthritis persists and if progress to ankylosing spondylitis is suspected. Sulphasalazine is the best-studied DMARD in ReA [Toivanen and Toivanen 2004]. Biological response modifiers have been found to be very effective in SpA [De Keyser *et al.* 2003]. There are also some case reports on the efficacy of infliximab in severe acute ReA.

Antibiotic treatment of the triggering infection is only recommended if the presence of infection can still be identified at the onset of arthritis. In enteroarthritis, antibiotic treatment has not been shown to influence the course of ongoing ReA. In patients with *Chlamydia trachomatis* triggered ReA, treatment with lymecycline resulted in faster recovery [Lauhio *et al.* 1991].

Prognostic factors

The prognosis of ReA is generally considered good. Most of the patients recover within a year. However, recurrences triggered by new infections are frequent. Recurrences and chronic development are more common in HLA-B27 positive patients. Persistent or recurrent urogenital infection or a chronic inflammatory focus in the gut may contribute to the progression of acute ReA to chronic SpA. Male gender and positive family history for SpA or ankylosing spondylitis are also considered adverse prognostic factors [Toivanen and Toivanen 2004].

AIMS OF THE STUDY

The overall purpose of the present study was to evaluate markers of immune activation as predictors of remission in rheumatoid arthritis (RA) and reactive arthritis (ReA). The specific aims of the study were:

1. Evaluation of sE-selectin (I, II, III), procalcitonin (II), and CD11b (II) as markers of systemic inflammation in rheumatoid arthritis (I, II) and reactive arthritis (II)

2. Evaluation of lymphocyte activation measured by sIL-2R in patients with early (III) and refractory (IV) RA

3. Evaluation of lymphocyte activation measured by sIL-2R in patients with acute ReA (V)

MATERIALS AND METHODS

Subjects

The present study comprises a total of 282 patients with RA and 54 patients with ReA. The characteristics of the patients are shown in Table 3. All study protocols were approved by the local ethics committees, and the patients gave written informed consent to participate.

The 25 patients with blood-culture positive sepsis serving as reference subjects (study II) were recruited at the Emergency Department, Helsinki University Central Hospital, Helsinki, Finland, for a previously published study [Takala *et al.* 1999b]. The criteria for septic shock was as defined by Bone *et. al.* [1992].

The 51 (study I) and 67 (study II) healthy controls were hospital staff members who were not taking medication and had no clinical signs of infection.

Study I comprised consecutive patients with early RA taking part in an ongoing prospective study carried out at the Department of Rheumatology, University Hospital, Lund, Sweden [Eberhardt *et al.* 1990]. The patients fulfilled the 1958 American Rheumatism Association (ARA) criteria for definite or classical RA [Ropes *et al.* 1959], duration of joint symptoms was less than 24 months, and patients were at least 18 years old on presentation. The 85 patients included in the present study had serum samples available at entry and at 1, 2, 3, 4, and 5 years.

The patients with active disease were offered treatment with DMARDs together with low dose (≤10 mg daily) prednisolone, if clinically indicated. Active disease was considered as the presence of six or more swollen joints and at least two of the following features: (a) ≥9 tender

Table 3. Characteristics of the patients

Study	Diagnosis	n	Female, n (%)	Age, years, mean (range)	Duration of symptoms, mean (range)
I	RA	85	54 (64%)	52 (18-78)	11.4 (SD 6.6) months
II	ReA	28	10 (36%)	37 (19-58)	3.8 (0.1-12) months
	RA	16	14 (88%)	45 (29-68)	5.9 (2-12) months
III	RA	157	110 (70%)	47 (20-65)	8.2 (2-23) months
IV	RA	24	15 (63%)	55 (34-76)	14.3 (4-32) years
V	ReA	26	18 (67%)	46 (18-82)	6.8 (0-13) weeks

n, number of patients; SD, standard deviation; RA, rheumatoid arthritis; ReA, reactive arthrits.

joints, (b) morning stiffness of ≥45 minutes, or (c) an ESR of ≥28 mm/hour. During the follow-up, 51 patients (60%) received DMARDs and 14 patients (16%) received prednisolone.

Study II. The patients with RA and ReA were recruited at the Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland. They had been referred to the hospital for acute joint symptoms.

The patients with RA fulfilled the American College of Rheumatology criteria for RA [Arnett *et al.* 1988] and the duration of symptoms was less than 12 months. At examination, the patients had previously received no DMARD treatment.

The patients with ReA had acute mono- or oligoarthritis, and had to have microbiological or serological evidence of preceding infection or a history of infection prior to joint symptoms. The pathogen was identified in 18 patients (*Salmonella*, n=8; *Yersinia*, n=4; *Campylobacter*, n=2; *Chlamydia trachomatis*, n=2; *Chlamydia pneumoniae*, n=2). The remaining patients had a history of enteritis (n=9) or urethritis (n=1).

Study III. The FIN-RACo (FINnish Rheumatoid Arthritis Combination therapy) trial is a nation-wide multi-center, randomized, open parallel-group trial on patients with early RA comparing the efficacy and tolerability of treatment with either a combination of three DMARDs (initially sulfasalazine, methotrexate, and hydroxychloroquine) and prednisolone (COMBI), or monotherapy with a single DMARD (initially sulphasalazine) with or without prednisolone (SINGLE) [Möttönen *et al.* 1999]. The present study included 157 patients (81 COMBI and 76 SINGLE) whose baseline serum samples were available.

The inclusion criteria were: 1) fulfillment of American College of Rheumatology (ACR) criteria for RA [Arnett *et al.* 1988], 2) age 18-65 years, 3) duration of symptoms <2 years, and 4) active disease with ≥3 swollen joints, and at least 3 of the following: (a) erythrocyte sedimentation rate (ESR) of ≥28 mm/hour or C-reactive protein (CRP) level of ≥19 mg/liter, (b) morning stiffness of ≥29 minutes, (c) >5 swollen joints, or (d) >10 tender joints.

Study IV comprises 24 patients with chronic active RA. The patients had failed all conventional DMARDs. A total of 19 patients (79%) were on low-dose (≤15 mg/day) prednisolone at the start of infliximab. At the start of infliximab, the patients were on various DMARDs (Table 4). Despite that, the patients had active disease. After starting infliximab, DMARD treatment was continued with minor modifications during the study period. The patients received infliximab infusions (3-4 mg/kg) at entry, and at 2 weeks, 6 weeks, 14 weeks, and 22 weeks.

Study V. A prospective population-based study of the incidence of inflammatory joint diseases was carried out between May 1999 and May 2000 in Kronoberg county, Sweden

Table 4. DMARDs used at entry by the patients in study IV.

Therapy	n (%)
Single therapy	15 (62)
Methotrexate (MTX)	7 (29)
Leflunomide (LEF)	6 (25)
Azathioprine (AZA)	1 (4)
Podophyllotoxine (CPH82)	1 (4)
Combination therapy	9 (38)
MTX + LEF	1 (4)
MTX + CPH82	1 (4)
MTX + hydroxychloroquine (HCQ) + CPH82	2 (8)
MTX + HCQ + sulphasalazine (SSZ) + cyclosporine A	1 (4)
AZA + gold sodium thiomalate (GST)	1 (4)
AZA + SSZ	1 (4)
CPH82 + HCQ	1 (4)
HCQ + auranofin	1 (4)

n, number of patients

[Soderlin *et al.* 2002]. All patients with the final diagnosis of ReA (n=26) were selected for the present study. ReA was defined as an inflammatory joint disease either preceded by a history of infection less than 2 months from the onset of joint symptoms and verified by cultures or positive serology, or, in the absence of history of infection, by cultures or serology alone. The pathogen was identified in 20 patients (*Campylobacter*, n=15; *Yersinia* and *Chlamydia trachomatis*, n=1; *Salmonella* and *Campylobacter*, n=1; *Campylobacter* and *Streptococcus pyogenes*, n=1). The remaining patients had a history of gastroenteritis (n=1), urethritis (n=1), respiratory tract infection (n=4), or staphylococcal soft tissue infection (n=2).

Follow-up and outcome evaluation

The patients were seen by a rheumatologist at each study visit. A comprehensive medical history was obtained and a throrough physical examination was conducted. At entry, clinical laboratory tests were done as necessary for diagnosis and evaluation of disease activity. During study visits, clinical examinations and appropriate laboratory samples were performed for the evaluation of disease activity (Table 5).

In study I, joint inflammation was assessed using the active joint count, defined as the number of joints that were swollen and, in addition, tender on palpation or painful on motion. The 50 joints evaluated included all joints from the Ritchie index [Ritchie *et al.* 1968], except for the neck and the subtalar joints. Functional status was evaluated with a Swedish version of the Stanford Health Assessment Questionnaire (HAQ) disability index [Ekdahl *et al.* 1988], where physical function is evaluated with a questionnaire completed by the patient. A score describing disability on a scale of 0-3 is calculated based on the answers. The findings in

Table 7. Timing for evaluation of immune activation (sE-selectin, procalcitonin, CD11b, sIL-2R), and clinical, routine laboratory, and radiological findings.

Study	At entry	6 weeks	22 weeks	6 months	1 year	2 years	3 years	4 years	5 years
I	*sE-selectin*				*sE-selectin*	*sE-selectin*	*sE-selectin*	*sE-selectin*	*sE-selectin*
	Symptoms				Symptoms	Symptoms	Symptoms	Symptoms	Symptoms
	Laboratory				Laboratory	Laboratory	Laboratory	Laboratory	Laboratory
	Radiology								Radiology
II	*CD11b*								
	Procalcitonin								
	sE-selectin								
III	*sIL-2R*								
	sE-selectin			Remission					
IV	*sIL-2R*	ΔDAS28	ΔDAS28						
V	*sIL-2R*			Remission					

Symptoms, clinical evaluation of joint inflammation; Laboratory, evaluation of routine laboratory tests; Radiology, radiographs of hands and feet; Remission, evaluation of remission (III: ACR preliminary criteria with modifications, V: absence of joint symptoms); ΔDAS28, change in Disease Activity Score (28-joint count) since entry

radiographs of hands and feet were scored using the Larsen method [Larsen *et al.* 1977], which employs reference radiographs depicting each joint at five progressive stages of destruction. They are used to calculate a total score of 0-200, depicting radiological joint destruction.

In studies I and III, remission was evaluated using the ACR preliminary criteria for remission [Pinals *et al.* 1981]. In both studies the fatigue criteria was omitted, in study III also the duration criteria. In study V, remission was defined as the absence of swollen and tender joints. In study IV the DAS28 disease activity score [Prevoo *et al.* 1995] was calculated using the formula

$$DAS28 = 0.56 \cdot \sqrt{TJC28} + 0.28 \cdot \sqrt{SJC28} + 0.70 \cdot \ln(ESR) + 0.014 \cdot GH$$

where TJC28 and SJC28 are 28-joint tender and swollen joint counts, ESR is erythrocyte sedimentation rate, and GH is patient's evaluation of general health on a visual analogue scale (VAS).

Blood samples

Serum samples were obtained by laboratory personnel, and stored at -20°C (I, II, III, V). In study IV, blood EDTA plasma was obtained by centrifugation. The samples were stored at -20°C for 12-14 years (I), less than 1 year (II), 5-7 years (III), 2 years (IV), and 3-4 years (V) before measuring markers of immune activation.

To obtain samples for flow cytometry (II), blood was collected into a polystyrene tube (Falcon No. 2058, Becton-Dickinson Labware, Lincoln Park, NJ, USA), supplemented with 500 µl of pyrogen-free citrate (113 mmol/l; Baxter Healthcare Ltd, Thetford, Norfolk, United Kingdom) and prechilled at 0°C. The sample was incubated on ice, and the buffy-coat layer was collected and retained on ice until cell labelling.

Markers of immune activation

sE-selectin levels in serum (I-III) were measured using a commercially available ELISA kit (Bender MedSystems Diagnostics GmbH, Vienna, Austria). The detection limit was 1.6 ng/ml, with intra- and inter-assay coefficients of variation (CV) of 5.4% and 6.0%, respectively. Measurements were carried out according to the manufacturer's instructions. All samples were measured at least in duplicate. Serial dilutions of recombinant sE-selectin were assayed on each microplate and linear regression was used to construct a standard curve for calculating the sE-selectin levels of the samples.

sIL-2R levels in serum (III, V) or plasma (IV) were measured by the Immulite automated immunoassay analyzer (DPC, Los Angeles, California, USA). The detection limit was 10 IU/ml, with intra- and inter-assay CV of 3.3% and 7.2%, respectively. One measurement was taken from each sample. The results were automatically calculated using stored reference curves.

CD11b expression on neutrophils and CD14-positive monocytes (II) was determined by three-colour flow cytometry [Repo *et al.* 1993]. Aliquots of the buffy-coat cell suspension were double-labelled with pretitrated amounts of fluorescein-isothiocyanate (FITC)-conjugated CD14 (mouse anti-CD14 IgG2b) and phycoerythrin (PE)-conjugated CD11b (mouse anti-CD11b IgG2a) or a corresponding control (PE-conjugated mouse anti-keyhole limpet haemocyanin IgG2a) antibody (Becton Dickinson, San Jose, California, USA). Contaminating erythrocytes were lysed with ice-cold fluorescence activated cell sorting (FACS) lysing solution (Becton Dickinson). Leucocytes were suspended in 0.5% formaldehyde and stained with LDS-751 (Exciton, Dayton, Ohio, USA), a nucleic acid dye.

A FACScan flow cytometer (Becton Dickinson) and LYSYS II software served for the acquisition and analysis of the data. Two separate data sets, one for neutrophils (5×10^3 LDS-751-positive events) and another for monocytes (10^3 CD14-positive events), were acquired for each specimen. CD11b expression is reported in relative fluorescence units (RFU), i.e. as the median channel of the positively fluorescent cell population. In all experiments, >95% of neutrophils and monocytes were CD11b positive.

For each series of experiments, a control blood sample from a healthy volunteer was processed in parallel with patient samples. The flow cytometer settings and spectral compensations remained unchanged during the study period, and a new lot of each monoclonal antibody was compared with the old lot.

Procalcitonin levels in serum (II) were measured by an immunoluminometric assay (LUMItest PCT, BRAHMS Diagnostica, Berlin, Germany). Functional assay sensitivity was 0.3 ng/ml. According to the manufacturer, the intra- and inter-assay CV are 6%-10% within clinically relevant PCT levels. Standards of recombinant PCT were assayed in order to construct a standard curve for calculating the PCT levels of the samples.

Routine laboratory tests, such as erythrocyte sedimentation rate (I-V), serum C-reactive protein (I, II, IV, V), rheumatoid factor (I-V), and HLA-B27 (II, V) were carried out using standard methods in conjunction with the study visits.

Data analysis

Study I. The results are given as median or mean and interquartile range (IQR), range or standard deviation. Individual clinical and laboratory variables of each patient were plotted at one-year intervals, and the area under the curve (AUC) was calculated using the trapezoidal rule approximation method and standardised by the length of the study. The patients were divided into tertiles based on their sE-selectin AUC values and Jonckheere's test (a non-parametric test for trend) was used to evaluate within the tertiles of sE-selectin AUC values the ordered alternative hypothesis of the AUC values of the other clinical and laboratory variables or the changes in Larsen score. The χ^2-test was used for categorical variables. The patients were also divided into tertiles based on their baseline sE-selectin levels. The outcome measures were categorised using the median as the cut-off point. Odds ratios (OR) and associated 95% confidence intervals (CI) were calculated using both univariate and multivariate logistic regression analysis with robust variance estimates. Sex, age at onset, rheumatoid factor, shared epitope, and tertiles of baseline sE-selectin were entered as covariates. The Mann-Whitney test was used to assess the differences in the serum sE-selectin levels of patients and control subjects. The Wilcoxon test was used to evaluate the differences in clinical, laboratory, and radiological findings at entry and after follow-up.

Study II. The results are given as median and range. Statistical comparisons between subject groups were performed with the Kruskal-Wallis analysis of variance. *Post hoc* analysis was performed with the Mann-Whitney test, with the Bonferroni correction for multiple comparisons.

Study III. The results are given as mean and 95% CI or standard deviation (SD), or median and IQR. Receiver operating characteristic (ROC) curves were calculated to define the optimal cut-off point for the sIL-2R test in predicting remission. Confidence intervals of area under ROC curves were estimated using the bootstrap bias-corrected accelerated method. Equality of area under ROC curves were tested using an algorithm suggested by DeLong, DeLong and Clarke-Pearson [DeLong *et al.* 1988]. The optimal cut-off value was defined as the level with the greatest sum of sensitivity and specificity. OR with 95% CI for prediction of remission by baseline demographic, clinical and laboratory variables were calculated using multivariate logistic regression analysis.

Study IV. The results are given as mean change and 95% CI, mean and range or SD, or median and range. Pearson correlation coefficient was calculated for serum sIL-2R level and baseline DAS28 score. The changes in DAS28 scores, with 95% CI, were calculated. The p-values for changes in DAS28 scores were calculated by the t-test. The predictive value of baseline sIL-2R level for change in DAS28 score at 6 and 22 weeks was calculated by robust regression analysis.

Study V. The results are given as mean and range. Statistical comparison between the groups was made using permutation test with general scores. The normality of variables was evaluated by the Shapiro-Francia test. However, as the distribution of sIL-2R levels was skewed, bootstrap estimation was used to derive the 95% confidence interval.

RESULTS AND DISCUSSION

sE-selectin as a marker of systemic inflammation in early RA (I)

Table 6 shows the clinical, laboratory, and radiological findings of the patients. Despite the favourable clinical course, the joint destruction progressed. The median change (IQR) of the Larsen score during follow-up was 30 (6-55) in the whole patient group.

Table 7 presents the AUC values of clinical and laboratory findings according to the tertile distribution of sE-selectin AUC values. Serum CRP levels, peripheral blood leukocyte counts, and active joint counts all had a statistically significant monotonic increasing association with serum sE-selectin level AUC value tertiles.

In this prospective study, serum sE-selectin levels were associated with markers of systemic inflammation. Previous studies failed to find such associations [Blann *et al.* 1995, Littler *et al.* 1997, Salih *et al.* 1999]. However, these other studies were cross sectional and examined patients with chronic RA. There was also an association between sE-selectin levels and the joint damage process.

Table 8 presents the outcome measures according to the tertile distribution of sE-selectin AUC values. The functional outcome (HAQ score at the end of the five-year follow up) had a monotonic increasing association with the sE-selectin levels. HAQ score AUC values were

Table 6. Clinical, laboratory, and radiological findings at entry and after five year follow-up.

	At entry		After 5 years		
Variables	n	Median (IQR)	n	Median (IQR)	p
Active joint count (0-50)	83	7 (3-12)	82	2 (0-5)	<0.001
HAQ score (0-3)	81	0.8 (0.5-1.2)	81	0.9 (0.4-1.3)	0.060
Serum sE-selectin level (ng/ml)	85	35.4 (26.4-49.0)	85	25.6 (21.2-36.2)	<0.001
Serum CRP level (mg/l)	68	11 (0-41)	76	3 (0-28)	0.213
ESR (mm/h)	83	27 (15-45)	79	24 (10-41)	0.789
WBC ($\times 10^9$/l)	75	8.1 (6.1-10)	82	6.2 (5.5-7.6)	<0.001
Platelet count ($\times 10^9$/l)	75	241 (215-317)	80	225 (175-255)	0.001
Haemoglobin level (g/l)	83	122 (114-133)	82	128 (119-138)	0.025
Larsen score (0-200)	83	7 (3-13)	82	43 (14-64)	<0.001

n, number of patients; IQR, interquartile range; HAQ, Health Assessment Questionnaire; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count

not associated with sE-selectin AUC values. The progression of radiological joint destruction, expressed as the change in Larsen scores, was also associated with the sE-selectin levels.

Table 9 shows the results of the multivariate logistic regression analysis of the predictive value of baseline sE-selectin tertiles for functional and radiological outcome. The patients with baseline sE-selectin levels in the third tertile had an increased risk of having an above median HAQ score at five years.

Age, sex, presence of rheumatoid factor or shared epitope, achievement of remission, and use of DMARDs or oral corticosteroids were not associated with the sE-selectin AUC values or with the baseline sE-selectin levels.

Increased circulating sE-selectin levels might maintain low level microvascular inflammation, which may in time result in systemic inflammatory tissue injury. However, in this study on patients with early RA with relatively few cardiovascular complications, comorbidity was not associated with the sE-selectin levels. This is in contrast with previous findings, which showed that raised levels of von Willebrand factor, another marker of endothelial activation, were predictive of thromboembolic complications in patients with chronic RA [Wallberg-Jonsson *et al.* 1993].

Table 7. Clinical and laboratory findings (AUC values) according to tertile distribution of serum sE-selectin AUC values

	Serum sE-selectin AUC, tertiles						
	First		Second		Third		
Variables, AUC	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	p
Active joint count (0-50)	26	4 (2-6)	27	4 (2-6)	27	6 (3-8)	0.019
Serum CRP level (mg/l)	22	10 (0-19)	23	7 (3-25)	23	32 (12-62)	0.012
ESR (mm/h)	24	27 (14-39)	28	23 (17-33)	28	36 (20-59)	0.114
WBC ($\times 10^9$/l)	22	6.6 (6.0-7.5)	25	7.1 (6.0-8.5)	25	8.4 (6.3-9.3)	0.037
Platelet count ($\times 10^9$/l)	22	233 (214-255)	25	234 (216-279)	25	263 (226-312)	0.058
Haemoglobin level (g/l)	26	127 (122-131)	28	127 (118-135)	28	126 (113-139)	0.522

AUC, area under curve; n, number of patients; IQR, interquartile range; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count

Table 8. Functional and radiological outcome measures according to tertile distribution of serum sE-selectin AUC values

	Serum sE-selectin AUC, tertiles						
	First		Second		Third		
Variables	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	p
HAQ score at 5 years	27	0.8 (0.3-1.0)	28	1.0 (0.7-1.3)	26	1.1 (0.5-1.5)	0.021
Change in Larsen score	27	28 (4-44)	27	18 (4-52)	28	46 (22-66)	0.038

n, number of patients; IQR, interquartile range; HAQ, Health Assessment Questionnaire

Table 9. Odds of above median HAQ score at 5 years (cut-off point 0.9) and change in Larsen score (cut-off point 29) in multivariate logistic regression analysis

Covariates	HAQ score at 5 years OR (95% CI)	Change in Larsen score OR (95% CI)
Sex (female)	5.51 (1.81 to 16.8)	1.26 (0.45 to 3.50)
Age at onset	1.02 (0.97 to 1.07)	0.97 (0.93 to 1.01)
Rheumatoid factor	1.09 (0.33 to 3.57)	2.03 (0.66 to 6.30)
Shared epitope		
None	1	1
Single	0.41 (0.07 to 2.35)	0.74 (0.09 to 5.92)
Double	1.14 (0.21 to 6.23)	3.77 (0.43 to 33.26)
Baseline sE-selectin tertiles		
First	1	1
Second	2.45 (0.70 to 8.59)	1.17 (0.37 to 3.70)
Third	4.18 (1.15 to 15.22)	1.58 (0.43 to 5.80)

HAQ, Health Assessment Questionnaire; OR, odds ratio; CI, confidence interval

At study entry, the median sE-selectin levels of the patients (35.4 ng/ml, IQR 26.4-49.0, n=85) and healthy control subjects (42.8 ng/ml, IQR 28.5-53.5, n=51) were comparable. Four patients (5%) had a raised sE-selectin level at entry (range 81.6-105.6 ng/ml). Similar levels have been detected in another study of 13 patients with early RA [Veale *et al.* 1998] and also in studies of patients with longstanding RA [Blann *et al.* 1995, Carson *et al.* 1994, Voskuyl *et al.* 1995].

CD11b, procalcitonin and sE-selectin as markers of systemic inflammation in acute ReA and early RA (II)

The results show that peripheral blood neutrophil and monocyte surface density of CD11b molecules and serum levels of PCT and sE-selectin in healthy subjects and patients with ReA and RA are comparable, and in patients with sepsis are high (Table 10, Figure 4). Consequently, these markers may be helpful in discriminating acute ReA and early untreated RA from sepsis.

Although CD11b density on neutrophils has been reported as normal in patients with RA [Crockard *et al.* 1992, De Clerck *et al.* 1995, Felzmann *et al.* 1991, Lopez *et al.* 1995, McCarthy *et al.* 1992, McCarthy *et al.* 1992, Nielsen *et al.* 1999, Storgaard *et al.* 1996, Watson *et al.* 1993], reports of increased [Macey *et al.* 1993, Torsteinsdottir *et al.* 1999a] and decreased values also exist [Crocker *et al.* 2000, Jones *et al.* 1994]. These inconsistent

findings may derive from differences between patient populations or methodological differences.

Several reports exist of high monocyte CD11b density in RA patients [Higaki *et al.* 1992, Highton *et al.* 1995, Liote *et al.* 1996, McCarthy *et al.* 1992, Torsteinsdottir *et al.* 1999b]. Unlike in those of our study, patients in previous studies had advanced RA and received a range of DMARDs and corticosteroids. Also, isolation of phagocytes by density gradient centrifugation [Repo *et al.* 1993] and fixation procedures [McCarthy *et al.* 1994, Repo *et al.* 1993] modify CD11b expression.

In patients with ReA, less is known about phagocyte activation. In a recent study, monocytes in acute ReA were activated, as evidenced by high CD11b density and the presence of messenger RNA for proinflammatory cytokines [Kirveskari *et al.* 1999]. In the present study, monocyte CD11b expression in ReA patients and controls was comparable. Although the reason for this discrepancy is unknown, it may involve differences in sample handling. In the earlier study, blood samples were anticoagulated with heparin at room temperature, which may enhance phagocyte metabolism [El Habbal *et al.* 1995]. Our results, in agreement with Felzmann *et al.* [1991], also show that neutrophil CD11b expression in patients with acute ReA falls within the normal range.

Many of the sepsis patients had normal CD11b expression (Figure 4, A and B). In contrast, PCT levels of patients with RA and ReA did not overlap with those of the sepsis patients (Table 10; Figure 4, C), indicating that as a sepsis marker PCT was superior to CD11b. These results agree with previous results indicating that in infection-free patients with active RA, PCT levels are normal [Schwenger *et al.* 1998]. Although sE-selectin levels in the sepsis group were high, they overlapped with those in the RA and ReA groups (Table 10; Figure 4, D).

Table 10. Markers of systemic inflammation in patients with rheumatoid arthritis, reactive arthritis, sepsis, and healthy control subjects.

	RA		ReA		Sepsis		Controls	
Variables	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)
Neutrophil CD11b, RFU	16	53 (31-95)*	28	50 (29-88)*	25	94 (44-269)	67	50 (29-96)*
Monocyte CD11b, RFU	16	44 (20-64)*	28	40 (23-81)*	25	91 (27-238)	67	37 (16-80)*
Procalcitonin, ng/ml	10	0.3 (0.2-0.3)*	21	0.3 (0.2-0.4)*	23	11.7 (0.6-515.0)	12	0.3 (n.a.)*
sE-selectin, ng/ml	10	71.0 (36.0-145.0)*	20	53.5 (20.0-109.0)*	20	203.0 (61.0-713.0)	10	67.3 (20.0-109.0)*

*, $p<0.01$ compared to Sepsis group. RA, rheumatoid arthritis; ReA, reactive arthritis; n, number of patients; RFU, relative fluorescence unit; n.a., not applicable.

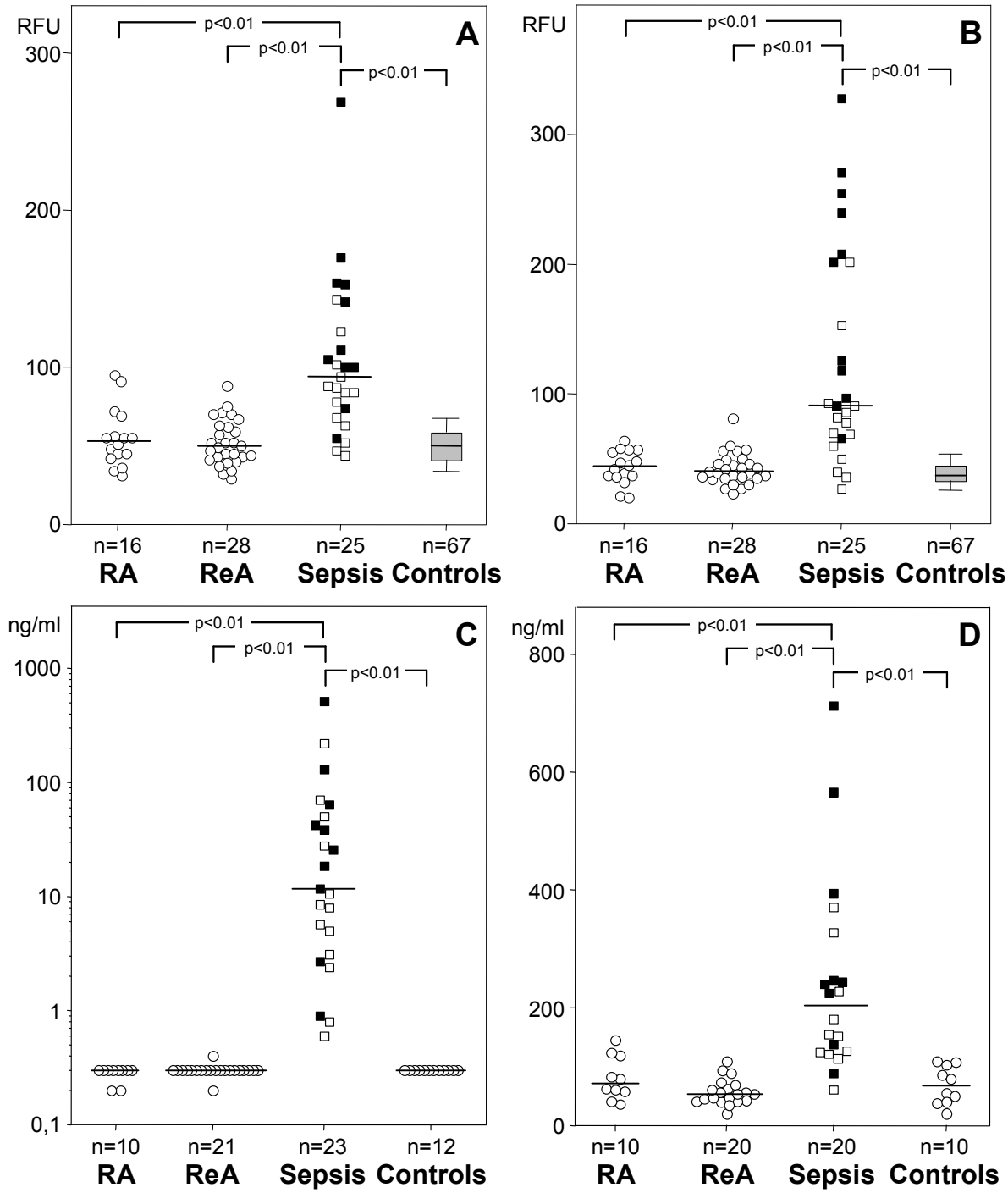

Figure 4. CD11b fluorescence intensity of neutrophils (A) and monocytes (B), together with serum procalcitonin (C) and sE-selectin (D) levels. Horizontal lines indicate group medians. In charts (A) and (B), lower and upper boundaries of the box indicate the 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles. Chart (C) is logarithmic scale. Abbreviations: RA, rheumatoid arthritis; ReA, reactive arthritis; n, number of patients. Symbols: open circles, arthritides or controls; open squares, sepsis; filled squares, septic shock.

sIL-2R and sE-selectin as predictors of remission in early RA (III)

At six months, 19 COMBI patients (23% [95% CI: 15 to 34]) and 7 SINGLE patients (9% [95% CI: 4 to 18]) (2 on prednisolone) were in remission. Among SINGLE patients, the mean baseline sIL-2R levels were 429 U/ml (95% CI: 366 to 614) in patients who entered remission and 737 U/ml (668 to 829) in patients who did not enter remission ($p<0.001$) (Figure 5). Among COMBI patients, the respective figures were 660 U/ml (570 to 802) and 739 U/ml (672 to 824) ($p=0.35$) (Figure 5).

COMBI therapy and baseline sIL-2R level <442 U/ml were the only significant predictors of remission after six months in logistic regression analysis (Table 11). Figure 6 shows the ROC curves of serum sIL-2R level for prediction of remission. Only SINGLE values differed significantly from the reference line i.e., AUC 0.50, $p=0.003$. The difference between AUC for SINGLE (0.86 [95% CI: 0.62 to 0.95]) and that for COMBI (0.57 [95% CI: 0.42 to 0.71]) was significant, $p=0.006$.

Thus, low baseline levels of sIL-2R (<442 U/ml) predict remission at 6 months in patients with active early RA treated with a single DMARD. The present finding is not in accordance with two previous studies in which the baseline sIL-2R levels failed to predict the clinical outcome of RA patients treated either with methotrexate [Polisson *et al.* 1994], or with sulfasalazine or parenteral gold [Merkel *et al.* 1996]. Unlike the present study, both studies included patients with longstanding RA, with mean disease duration more than 10 and 5 years, respectively. Such patients with a long disease duration may be less prone to spontaneous or drug induced remissions, which occurs in 10 to 30 percent of patients with early RA [Ollier *et al.* 2001].

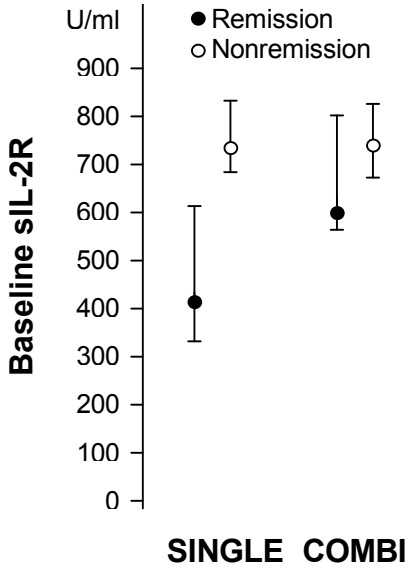

Figure 5. Baseline sIL-2R levels in single disease-modifying antirheumatic drug group (SINGLE) and combination drug group (COMBI) according to remission after 6 months. Dots denote group means and whiskers their 95% confidence intervals.

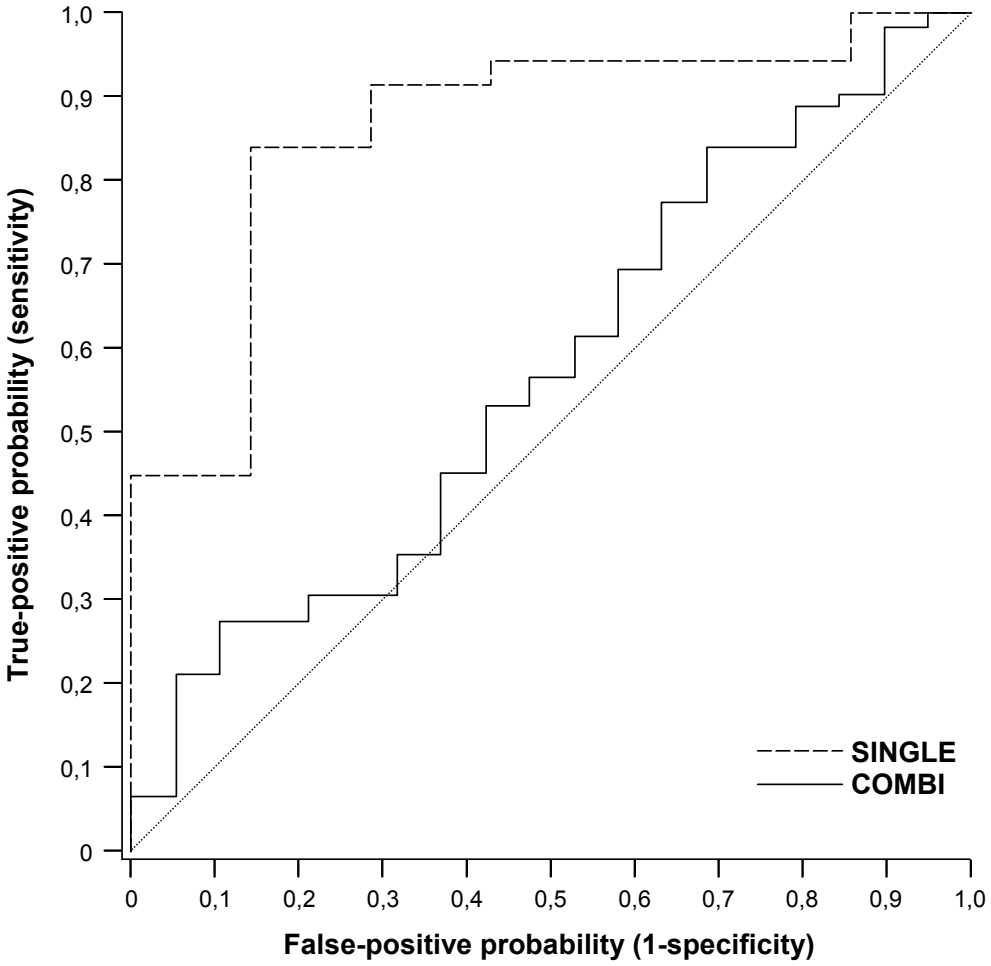

Figure 6. ROC curves of baseline sIL-2R level for prediction of remission at six months in SINGLE and COMBI groups. Diagonal dotted line represents reference line ("line of no information").

Table 11. Logistic regression analysis for the odds to reach remission after six months

Variable at beginning of study	OR (95% CI)
Combination strategy	4.4 (1.6 to 12.2)
sIL-2R level <442 U/ml	4.7 (1.4 to 15.2)
Female sex	1.0 (0.3 to 3.1)
Age in years	1.0 (0.9 to 1.1)
Disease duration in months	1.0 (0.9 to 1.1)
Rheumatoid factor present	1.2 (0.4 to 3.7)
Erythrocyte sedimentation rate	1.0 (0.9 to 1.1)
Swollen joint count	1.0 (0.9 to 1.1)
Tender joint count	0.9 (0.9 to 1.0)

OR, odds ratio; CI, confidence interval.
Odds ratios are adjusted for all other variables in the model.

The respective median (IQR) sE-selectin levels in SINGLE for remission and for nonremission subgroups were 33.7 ng/ml (26.9 , 52.2) and 40.8 ng/ml (26.2 , 55.2). The corresponding values in COMBI were 43.2 ng/ml (32.2 , 56.4) and 37.9 ng/ml (27.8 , 50.7). The AUC values for SINGLE and COMBI were comparable, 0.58 (95% CI: 0.34 to 0.73) vs. 0.45 (95% CI: 0.31 to 0.61). Neither group differed significantly from the reference line (AUC 0.50). In conclusion, baseline sE-selectin used as marker of systemic inflammation did not predict early remission in patients treated actively with DMARDs.

sIL-2R as predictor of treatment response in refractory RA (IV)

The mean serum sIL-2R level was 621 U/ml (SD 325) and mean DAS28 score was 6.3 (SD 1.0). The serum sIL-2R level did not correlate with the baseline DAS28 score, r=0.24 (95% CI: -0.18 to 0.58). This is in agreement with previous studies which have shown poor [Beckham *et al.* 1992, Franke *et al.* 1997, Salaffi *et al.* 1995] or no [Tebib *et al.* 1995] correlation between serum level of sIL-2R and clinical activity of RA.

There was a distinct clinical response to infliximab. At 6 weeks, DAS28 scores improved (mean score 3.7, SD 1.7), with a mean change of –2.53 (95% CI: -3.08 to -1.95, $p<0.001$). This change was predicted by baseline sIL-2R level (Table 12; Figure 7, a) but not by baseline DAS28 score or infliximab dose. Given that the serum sIL-2R level serves as a marker to T-cell activation [Rubin and Nelson 1990], our results suggest that enhanced T-cell activation is related to delayed response to the infliximab therapy.

At 22 weeks, the mean DAS28 score was 4.0 (SD 1.2), and the mean change from baseline was –2.26 (95% CI: -2.75 to -1.77, $p<0.001$). This change was predicted by neither baseline sIL-2R level (Table 12; Figure 7, b) nor baseline DAS28 score or infliximab dose. The finding agrees with the previous studies of refractory RA patients treated with methorexate for 18 weeks [Polisson *et al.* 1994] or DMARD naive patients treated with sulphasalazine or parenteral gold for 37 weeks [Merkel *et al.* 1996], indicating no correlation between initial serum sIL-2R level and response to the therapy.

Table 12. Baseline-adjusted robust regression for baseline sIL-2R predicting change in DAS28 score after 6 and 22 weeks.

	DAS28 change from baseline			
	After 6 weeks		After 22 weeks	
Explanatory variables	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Baseline sIL-2R (per 100 U/ml)	0.2054 (0.0035 , 0.4073)	0.047	0.0152 (-0.1872 , 0.2176)	0.88
Baseline DAS28 score	0.0585 (-0.5327 , 0.6498)	0.84	-0.3067 (-0.8730 , 0.2595)	0.27
Infliximab dose (mg/kg)	0.1313 (-0.3719 , 0.6346)	0.59	0.0185 (-0.4515 , 0.4885)	0.94
Constant	-4.995 (-8.708 , -1.2823)		-0.1731 (-3.220 , 2.873)	

CI, confidence interval; DAS28, Disease Activity Score (28-joint count)

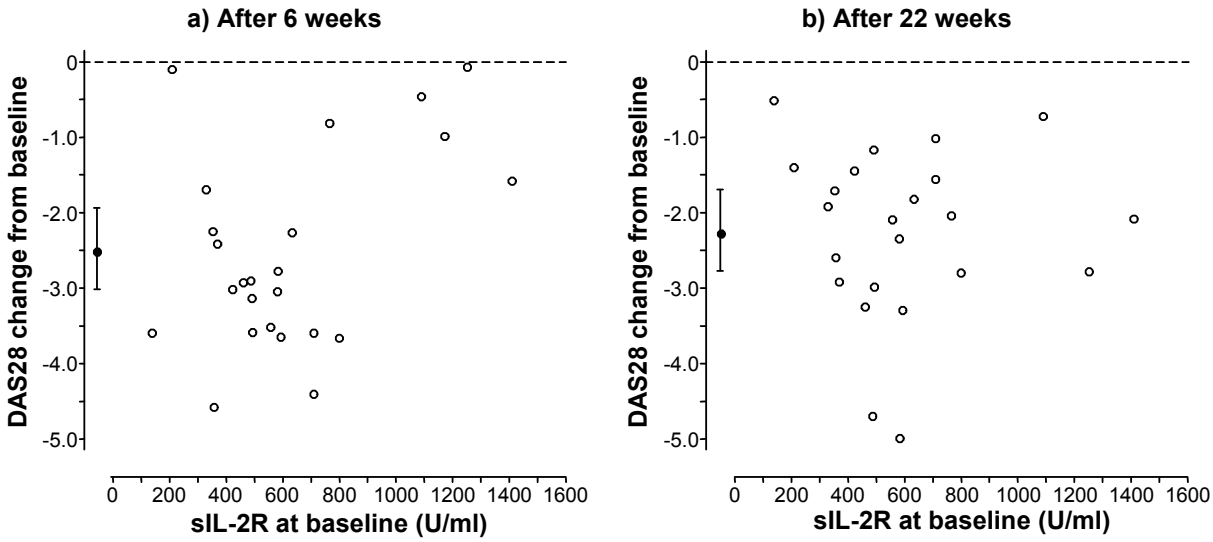

Figure 7. Baseline serum sIL-2R versus change in DAS28 score from baseline to 6 weeks and baseline to 22 weeks. Symbols: filled circle, mean; whiskers, 95% confidence interval.

sIL-2R as predictor of remission in acute ReA (V)

A total of 17 patients (65%) were in remission at 6 months, and 9 patients (35%) still had joint symptoms. At baseline, the patients in remission at 6 months had higher sIL-2R levels (Figure 8), 891 U/ml (95% CI: 658 to 1123) versus 501 U/ml (95% CI: 436 to 566), p=0.022. The baseline sIL-2R level was 528 U/ml (95% CI: 416 to 639) in HLA-B27 positive (n=6) and 824 U/ml (95% CI: 618 to 1030) in HLA-B27 negative (n=20) patients, p=0.14. Thus, a high serum sIL-2R level in a patient with early acute ReA seems to predict remission by 6 months. The only previous study of sIL-2R in patients with ReA [Steiner *et al.* 1995] did not examine the role of sIL-2R as a predictor of clinical outcome of acute ReA.

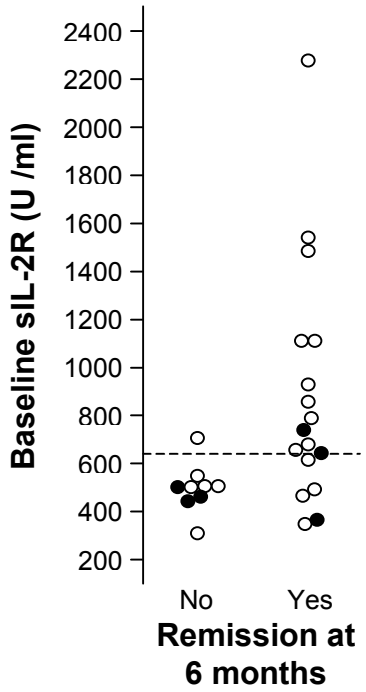

Figure 10. Baseline sIL-2R levels according to remission at 6 months. Symbols: filled circle, HLA-B27 positive; open circle, HLA-B27 negative; dotted line, median sIL-2R level in all patients.

GENERAL DISCUSSION

Limitations of the study

The studies were based on serum or plasma samples stored for up to 14 years at -20˚C. This was recognized while designing the studies, and both sE-selectin and sIL-2R chosen to be measured from stored samples are relatively stable molecules able to withstand even storage at room temperature for a limited period of time. Therefore it is unlikely that extended storage has substantially affected the results.

Another factor that may have an effect on levels of circulating markers is inadequate clearance due to renal disease or use of NSAIDs. The possible effect of these factors on the results cannot be excluded. In addition, the patients in study IV were on various DMARD prior to and during the study period, which may have affected the sIL-2R levels measured or response to infliximab treatment.

Validity of the results

The patients in study I were chosen from the cohort because they had a full set of serum samples available. That is, however, unlikely to have led to a bias. The other studies comprised consecutive patients. Thus, the patient samples are representative of appropriate patients seen in everyday rheumatological practice.

The common limitation of studies II, IV, and V is the relatively small number of patients, which should be taken into consideration while interpreting the results. However, is should be noted that compilation of such patient cohorts is rather arduous.

sE-selectin as a marker of systemic inflammation in early RA (I)

Other investigators [Newman *et al.* 1993] have shown a correlation between serum and synovial fluid concentrations of sE-selectin, and our findings might therefore give some support to the hypothesis that E-selectin participates in the pathogenic mechanisms in the rheumatoid joint. The synovial fluid levels are higher in RA than in non-inflammatory joint diseases [Koch *et al.* 1993, Newman *et al.* 1993]. Furthermore, expression of E-selectin molecules occurs in the rheumatoid synovial tissue [Elewaut *et al.* 1998, Kriegsmann *et al.* 1995] and seems to be associated with synovial inflammatory activity [Elewaut *et al.* 1998].

The sE-selectin levels at entry and during follow-up were associated with functional status after five years. The observed association can be explained, at least in part, by the proinflammatory properties of sE-selectin, which might promote joint inflammation and thereby compromise physical function of the patient. Furthermore, IL-1, the key mediator of systemic inflammation, causes fatigue through its effects on the central nervous system [Licinio and Wong 1997]. Fatigue affects the subjective experience of functional ability and is reflected in the HAQ score [Scharloo *et al.* 1999, Wolfe *et al.* 1996].

The sE-selectin levels, although within the normal range, may affect chronic inflammation and a slight increase may occur, for example in patients with clinically relevant atherosclerosis [Fassbender *et al.* 1999, Frijns *et al.* 1997]. Similarly, CRP levels, although within the normal range, have been shown to predict coronary events [Rifai and Ridker 2001]. Given that CRP and sE-selectin are both markers of systemic inflammation, the hypothesis can be proposed that sE-selectin levels within the normal range may also reflect smouldering inflammation. The clinical relevance of sE-selectin levels is an entirely different issue, and the results of this study show that the use of sE-selectin measurements in the clinic cannot be advocated owing to their inadequate predictive value.

CD11b and procalcitonin as markers of systemic inflammation in acute ReA and early RA (II)

In studies comparing CD11b expression on peripheral blood and synovial fluid phagocytes in RA patients, higher expression levels have consistently been found on synovial fluid cells [Crockard *et al.* 1992, De Clerck *et al.* 1995, Felzmann *et al.* 1991, Lopez *et al.* 1995]. The findings of normal CD11b expression on circulating phagocytes in peripheral blood may result from the tendency of activated cells leaving the circulation and homing to the inflamed synovium. It is also possible that the activation of phagocytes resulting in elevated CD11b expression occurs only after the cells have entered the inflamed synovium.

In sepsis, bacterial components, such as LPS are considered to cause systemic inflammation. Patients with ReA have been shown to carry bacterial LPS in circulating phagocytes [Granfors *et al.* 1998]. Such LPS molecules, however, are not necessarily proinflammatory. Indeed, phagocytes can deacylate LPS which abolishes many of the inflammatory properties of LPS [Repo *et al.* 1994]. There are data showing that monocytes may generate PCT [Oberhoffer *et al.* 1999]. LPS could stimulate ReA monocytes to produce increased amounts of PCT. However, the circulating PCT levels in ReA were found to be normal. Thus, despite the spreading of microbial antigens and systemic inflammation in ReA, the laboratory markers are different between ReA and sepsis patients.

sIL-2R as predictor of treatment response in early (III) and refractory (IV) RA

Low circulating sIL-2R level predicted remission at 6 months in patients with active early RA treated with a single DMARD (III). It is possible that in some patients with a low degree of T-cell activation even a single DMARD is able to induce remission, whereas combination therapy appears to be effective even in patients with high T-cell activation. It is also possible that low sIL-2R identifies the patients bound to enter spontaneous remission.

With respect to serum sIL-2R levels, the patients with RA refractory to DMARDs in study IV were similar to the DMARD naive patients in study III. Thus, low sIL-2R level can be a feature of early untreated RA or of patients with active disease despite treatment with various DMARDs. Low sIL-2R level may be a marker of a subtype of RA, notoriously known to be heterogeneous, and indicate a more rapid and favourable response to treatment. The findings of these studies raise the intriguing question whether the patients with high sIL-2R levels, denoting high lymphocyte activation, would be particularly suitable for treatment with CTLA4Ig [Kremer *et al.* 2003] or other drugs suggested to play via inhibition of T cell activation [Lorenz 2003].

sIL-2R as predictor of remission in acute ReA (V)

In patients with RA, low circulating sIL-2R levels predicted early response to treatment (III, IV). However, in patients with ReA, high sIL-2R levels were associated with good outcome (V). This discordance is probably related to the different immunopathogenesis of these diseases. RA is characterised by a Th1 immune response [Choy and Panayi 2001], whereas in ReA a Th2 type response is seen [Smeets *et al.* 1998]. It has been suggested that secretion of sIL-2R is associated with a Th1-type response [Berghella *et al.* 1998]. Thus, the high sIL-2R levels in the ReA patients with a favourable outcome could reflect beneficial Th1 immune activation aimed at eliminating the microbe. In patients with RA, however, high sIL-2R levels might reflect a harmful Th1 autoimmune response. Interestingly, low sIL-2R levels have been observed in patients with chronic spondyloarthropathy [Toussirot *et al.* 1994] where an impairment of T cell activation has been observed [Baeten *et al.* 2004], as an indirect evidence of the failure of immune response to induce remission.

In study V, HLA-B27 positive patients had especially low serum sIL-2R levels. HLA-B27 is a strong marker for severity and chronicity of ReA. In hospital-based series, most of the patients are HLA-B27 positive [Leirisalo *et al.* 1982]. The frequency is considerably lower in community-based series [Hannu *et al.* 2002], as also in the present study. HLA-B27 has been studied with respect to cytokine production and prognosis of ReA. Of patients with Chlamydia induced acute ReA, those with HLA-B27 had lower levels of IFN-γ in synovial fluid, which was associated with a chronic course of arthritis [Bas *et al.* 2003b]. Also, low

secretion capacities of TNF and IFN-γ by peripheral blood mononuclear cells by patients with acute ReA predicted the persistence of arthritis for more than 6 months [Braun *et al.* 1999]. Taken together, the results above raise a possibility that novel treatment modalities, such as immune stimulation with IFN-γ might enhance both microbial elimination and clinical recovery at least in some patients with acute ReA. Although the results in the present study are preliminary and need to be interpreted cautiously they suggest that sIL-2R aids to identify the ReA patients who might benefit from immune stimulation.

Future prospects

The results show that sIL-2R is a promising predictive marker in both RA and ReA. In order to further analyze the value of this marker, new prospective studies comprising larger numbers of patients are necessary. Also other predictive markers should be sought using traditional biochemical methods, such as in the present study, and also cutting-edge approaches, such as genomics and proteomics. The goal of the research should be to provide individual patients with arthritis tailored treatment with optimal efficacy, tolerability, and affordability.

CONCLUSIONS

Activation of vascular endothelium, reflected by circulating sE-selectin levels, was associated with joint inflammation and markers of systemic inflammation (C-reactive protein and white blood cell count) during a follow-up period of five years. Serum sE-selectin levels during follow-up were associated with functional and radiological outcome after five years as well, and baseline levels also predicted functional outcome (I).

Patients with acute ReA and early RA have normal CD11b expression levels on phagocytes, and normal procalcitonin and sE-selectin levels in serum (II). Elevated levels of these markers may be a sign of sepsis.

Low baseline level of circulating sIL-2R predicts remission at 6 months in patients with active early RA treated with a single DMARD (III) and rapid response to infliximab treatment in patients with refractory RA (IV).

High circulating sIL-2R level in acute ReA is a marker of good outcome (V), possibly reflecting a well functioning immune response to eradicate the triggering microbe.

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